ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF TENELIGLIPTIN IN ORAL SOLID DOSAGE FORM BY REVESRE PHASE CHROMATOGRAPHIC TECHNIQUE USING UHPLC

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MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

Submitted by

Ganesh Prabhu. K
Reg No : **261530653**

Under the guidance of

Institutional Guide

Dr.W.D.Sam Solomon, M.Pharm., Ph.d.,
Professor & Head
Department of Pharmaceutical Sciences
RVS College of Pharmaceutical Sciences ,
Sulur , Coimbatore – 641402

Industrial Guide

Mr. Gopinath P,
Manager of Quality control Department,
Malur,
Karnataka-563031

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DEPARTMENT OF PHARMACEUTICAL ANALYSIS,
R.V.S. COLLEGE OF PHARMACEUTICAL SCIENCES
SULUR, COIMBATORE - 641 402, TAMIL NADU.

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This is to certify that the dissertation work entitled "**Analytical method development and validation for the estimation of Teneligliptin in oral solid dosage form by revesre phase chromatographic technique using UHPLC**" is a bonafide work of **Ganesh Prabhu. K** carried Out in **Medopharm, Malur.** Under my guidance and supervision of **Mr**.**Gopinath**, for the partial fulfillment of the award of degree of Master of Pharmacy in **Pharmaceutical Sciences**, RVS college of Pharmaceutical Sciences, Sulur, Coimbatore, affiliated to The Tamilnadu Dr. M.G.R Medical University, Chennai.

Dr.W.D.Sam Solomon, M.Pharm., Ph.d.,

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Dr.R.Venkatanarayanan, M.Pharm., Ph.d.,

Date:

Place: **Coimbatore**

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Institutional Guide
Dr.W.D.Sam Solomon, M.Pharm., Ph.d.,
Professor & Head
Department of Pharmaceutical Sciences
RVS College of Pharmaceutical Sciences ,
Sulur , Coimbatore – 641402

Dr.R.Venkatanarayanan, M.Pharm., Ph.d.,
Principal.
RVS College of Pharmaceutical Sciences ,
Sulur , Coimbatore – 641402

Evaluation Certificate

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Name of the Candidate **:** **Ganesh Prabhu. K** ***(Reg No :*** **261530653)**

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Institution Name **:** RVS college of Pharmaceutical Sciences, Sulur, Coimbatore.

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GANESH PRABHU

(261530653)

1.0 INTRODUCTION

The pharmaceutical analysis is a branch of chemistry, which involves the series of process for the identification, determination, quantitation, and purification. This is mainly used for the separation of the components from the mixture and for the determination of the structure of the compounds. The different pharmaceutical agents are Plants, Minerals Microorganisms and Synthetic compounds[1]

Pharmaceutical analysis plays a vital role in the Quality Assurance and Quality control of bulk drugs. Analytical chemistry involves separation, identification, and determining the relative amounts of components in a sample matrix; Pharmaceutical analysis is a specialized branch of analytical chemistry that derives its principles from various branches of sciences like physics, microbiology, nuclear science, and electronics etc. Qualitative analysis is required before a quantitative analysis can be undertaken.

A separation step is usually a necessary part of both a qualitative and quantitative analysis. The result of typical quantitative analysis can be computed from two measurements. One is the mass or volume of sample to be analyzed and the second one is the measurement of some quantity that is proportional to the amount of analyte in that sample and normally completes the analysis.

The main steps that are performed during a chemical analysis are the following:

(1) sampling,

(2) field sample pretreatment,

(3) laboratory treatment,

(4) laboratory assay,

(5) calculations, and

(6) Results presentation.

Each must be executed correctly in order for the analytical result to be accurate. Some analytical chemists distinguish between an analysis, which involves all the steps, and an assay, which is the laboratory portion of the analysis[2]

The complete analysis of a substance consists of 5 main steps.

1. Sample preparation / Sampling
2. Dissolution of the sample,
3. Conversion of the analyte into a form suitable for measurement.
4. Measurement
5. Calculation and interpretation of the measurement

Need for pharmaceutical Analysis

1. New Drug Development.
2. Method Validation as for ICH Guidelines
3. Research in Pharmaceutical Sciences
4. Clinical Pharmacokinetic Studies

When promising results are obtained from explorative validation performed during the method development phase, then only full validation should be started. The process of validating a method cannot be separated from the actual development of method condition[3]

1.1 Chromatographic Techniques

In 1903 a Russian botanist Mikhail Tswett produced a colorful separation of plant pigments through calcium carbonate column. Chromatography word came from Greek language chroma = color and graphein = to write i.e. color writing or chromatography [4, 5]

During 1970's, most chemical separations were carried out using a variety of techniques including open-column chromatography, paper chromatography, and thin-layer chromatography. However, these chromatographic techniques were inadequate for quantification of compounds and resolution between similar compounds. During this time, pressure liquid chromatography began to be used to decrease flow through time, thus reducing purification times of compounds being isolated by column chromatography. However, flow rates were inconsistent, and the question of whether it was better to have constant flow rate or constant pressure was debated[6]. High pressure liquid chromatography was developed in the mid-1970's and quickly improved with the development of column packing materials and the additional convenience of online detectors. In the late 1970's, new methods including reverse phase liquid chromatography allowed for improved separation between very similar

compounds. By the 1980's HPLC was commonly used for the separation of chemical compounds. New techniques improved separation, identification, purification and quantification far above the previous techniques. Computers and automation added to the convenience of HPLC. Improvements in type of columns and thus reproducibility were made as such terms as micro-column, affinity columns, and Fast HPLC began to immerge. By the 2000 very fast development was undertaken in the area of column material with small particle size technology and other specialized columns. The dimensions of the General Introduction typical HPLC column are 100-300 mm in length with an internal diameter between 3-5 mm. The usual diameter of micro-columns, or capillary columns, ranges from 3 μm to 200 μm [7]. In this decade sub 2 micron particle size technology (column material packed with silica particles of < 2μm size) with modified or improved HPLC instrumentation becomes a popular with different instrument brand name like UPLC (Ultra Performance Liquid Chromatography) of Waters and RRLC (Rapid Resolution Liquid Chromatography) of Agilent.

Classification of Chromatographic Techniques

I] According to the nature of stationary and mobile phase

- Gas Solid Chromatography
- Gas Liquid Chromatography
- Solid Liquid Chromatography
- Liquid Liquid Chromatography

II] According to mechanisms of separation, chromatographic methods are divided into

1. Adsorption chromatography
2. Partition chromatography
3. Ion exchange chromatography
4. Ion pair chromatography
5. Size exclusion or gel permeation chromatography
6. Affinity chromatography
7. Chiral phase chromatography

1.2INTRODUCTION TO HPLC

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres.

HPLC allows the use of very small particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture. Its simplicity, high specify and wide range of sensitivity makes it ideal for analysis of many drugs in both dosage forms and in biological fluid.

1.2.1 TYPES OF HPLC TECHNIQUES

A. Based on modes of separation: There are two modes, normal phase mode and reverse phase mode.

i) Normal phase mode: In this mode**,** the stationary bed is strongly polar in nature (e.g., silica gel), and the mobile phase is nonpolar (such as n-hexane or tetrahydrofuran). Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

ii) Reveres phase mode: Reverse-phase chromatography is the inverse of Normal phase mode. The stationary bed is nonpolar (hydrophobic) in nature, while the mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. Here the more nonpolar the material is, the longer it will be retained. Non-polar compounds in the mixture will tend to form attractions with the hydrocarbon groups because of vanderwaals dispersion forces. Since most of the drugs and pharmaceuticals are polar in nature, they are not retained for a longer time and eluted faster which is advantageous. Different columns used are ODS (octadecylsilane), C_{18}, C_8, C_4 etc.

B. Based on elution technique

Isocratic separation: In this technique the same mobile phase combination is used throughout the process of separation, the same polarity or elution strength is maintained through the process.

Gradient separation: In this technique, a mobile phase combination of low polarity or elution strengths is used followed by gradually increasing the polarity or elution strengths.

C. Based on the scale of operation:-

Analytical HPLC: In this technique only analysis of the samples are done. Recovery of the samples for reusing is normally not done, since the sample used very low. Ex.µgm quantities.

Preparative HPLC: In this technique individual fraction of pure compounds can be collected using fraction collector. The collected samples are reused. Ex. Separation of few grams of mixtures by HPLC.

D. Based on the type of analysis

Qualitative analysis: It is done to identify the compound, detect the presence of impurity, to find out the number of components etc... This is done by using retention time values.

Quantitative analysis: It is done to determine the quantity of the individual or several components in a mixture. This is done by comparing the peak area of the standard and sample.

1.2.2 PRINCIPLE OF HPLC

The principle of separation in normal phase mode and reverse phase mode is adsorption and/or partition.

ADSORPTION

When a mixture of components is introduced in to a HPLC column they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the stationary phase travels slower. The component which has less affinity towards the stationary phase travels faster. Since no two components have the same affinity towards the stationary phase the components are separated.

PARTITION

When a mixture of compounds are dissolved in the mobile phase and passed through a column of liquid stationery phase, the component which is more soluble in the stationery phase travels slower. The component which is more soluble in the mobile phase travels faster. Thus the components are separated because of the differences in their partition co-efficient. No two components have the same partition co-efficient for a particular combination of stationery phase, mobile phase and other conditions.

1.2.3 INSTRUMENTATION OF HPLC

The main components of HPLC system are as follows

a) Mobile phase reservoir
b) pumps
c) Sample mixing units
d) Sample injectors
e) Column
f) Detectors.
g) Recorders and injectors

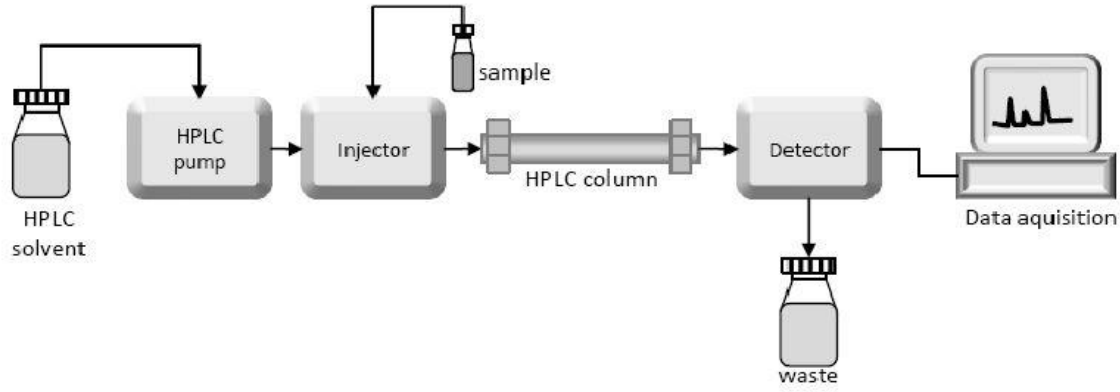


Figure No 1: Schematic diagram of HPLC

a) Mobile phase Reservoirs: The modern HPLC apparatus is equipped with one or more glass or stainless steel reservoirs each of which contains 200-1000ml of a solvent. The mobile phase are often equipped with a mean of removing dissolved gasses usually oxygen

or nitrogen. These bubbles cause band spreading, in addition they often interfere with performance of the detectors. Often the systems also contain a means of filtering dust and particulate matter from the solvent to prevent these particles from damaging the pumping systems or clogging systems. This treatment removes gases as well as suspended matter. A separation that employs a single solvent of constant composition is termed as isocratic elution. Frequently separation efficiency is greatly enhanced by gradient elution. Here two difference solvent systems that differ significantly on polarity are employed.

b) Pumps: The most important component of HPLC in solvent delivery systems is the pumps because its performance directly affects the retention time reproducibility and detector sensitivity. Three types of pumps each with its own set of advantages are encountered namely.

- Reciprocating pumps
- Displacement –type pumps
- Pneumatic pumps

Reciprocating Pumps

They usually consist of chamber in which the solvent is pumped by the back and forth motion of a motor drive piston. The two ball check valves which open and close alternately control the flow of solvent in to and (close alternately control the flow of solvent in to and) out of a cylinder track with the piston.

ii) Displacement Pumps

Displacement pump consist of large, syringe like chambers equipped with a plunger that is activated by a screw driven mechanism powered by a stepping metor. The output is pulse free.

iii) Pneumatic Pumps

The pneumatic pumps, the melphis contained in a compatible container housed in a vessel that can be pressurized by compressed gas. Pumps of this type are in expensive and pulse free, they suffer from limited capacity and pressure output as well as a

dependence of flow rate on solvent viscosity and column back-pressure. In addition they are not amiable to gradient elution and are limited to pressure less than about 2000psi.

c) Flow Control and Programming Systems

As part of their pumping systems, many commercial instruments are equipped with computer controlled devices for measuring the flow rate by determining the pressure drop across a restrictor located at the pump outlet.

d) Sample Mixing Units

Mixing unit is used to mix solvents in different proportions and pass through the column. They are Low pressure mixing chamber which uses helium for degassing solvents and High pressure mixing chamber does not require helium for degassing solvents.

e) Sample Injector

Often the limited factor in the precision of liquid chromatographic measurement lies in the reproducibility with which samples can be introduced in to columns packing. Exaggerated by band broadening which accompanies overloading columns. These volumes used must be minuscule a (Thus, the volumes used must be mini) of a ml to perhaps 500ml.It is convenient to be able to introduce the sample without depressurizing the system.

f) Columns

HPLC columns are ordinarily constructed from smooth-bore stainless steel tubing, although heavy-walled glass tubing is occasionally encountered.

Analytical columns

The majority of HPLC columns range in length from 10-30cm.Normally columns are straight with added length when needed, being gained by coupling two or more columns together occasionally called columns are encountered.

The inside diameter of liquid column is often 2-5mm.The most common particles size of packed columns is 5-10µm .the most common columns currently in use is one that is

15-25cm in length,4.6mm inside diameter ,and packed with 5µm particles. Columns of this type contain 4000-6000plates/meter.

Guard columns

The guard columns have very small quantity of adsorbent and improve the life of the analytical columns. It also acts as a pre-filter to remove particulate matter and any other material. Guard column has the same material as that of analytical column but does not contribute to any separation.

g) Column thermostats

For many applications close control of column temperature is not necessary and columns are appareled at combined temperature often. However better chromatograms are obtained by monitoring column temperature constant to the few degree centigrade. Most modern commercial instruments are now equipped with column heaters that control, column temperature to a few tenths of degree from near obtained to 100-150 ºc. Columns may also be fitted with water jackets fed from a constant temperature bath to give precise temperature control.

h) Column material

The columns are made up of either stainless steel glass polyethylene, PEEK (poly ether ether ketone). Most widely used are stainless steel which can with stand high pressure. Latest ones are PEEK columns whose length varies from 5-30cm, diameter ranges from 2-5mm and particle size from 1µ-20µ.Particle should be spherical and uniform in nature. Porous materials are used. Surface area of 1gm of stationary phase provides surface area ranging from 100-860 mts.

i) Functional group

The functional group present in stationary phase depends on the type of chromatographic separation in normal phase mode it contain following groups

- C_{18} –octadecylSilane (ODS) column
- C_8-octylcolumn

- C_4-butyl column
- CN-cyano column
- NH_2-amino column

j) DETECTORS

The function of detector in HPLC is to monitor the mobile phase as it emerges through the column

Types of detectors

Absorbance detector

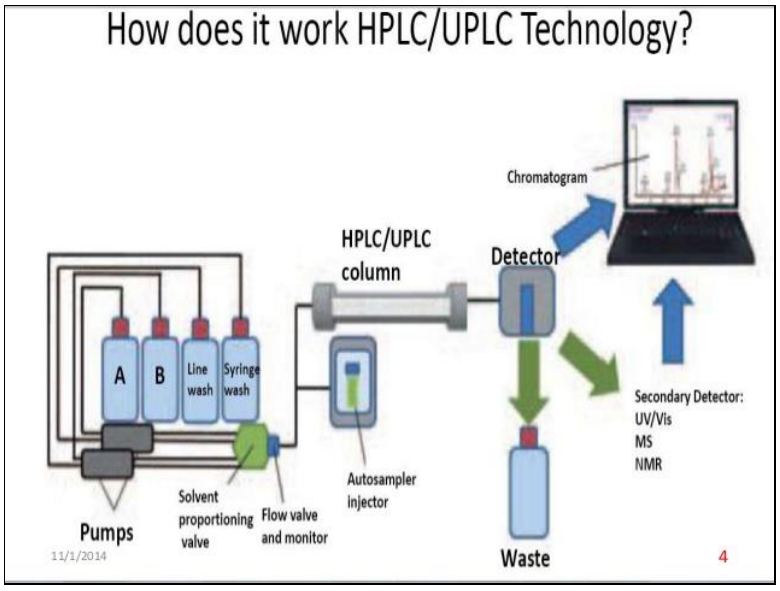
i) UV detector
ii) Refractive index detector.
iii) Flourimetric detector
iv) Conductivity detector
v) Amperometric detector
vi) PDA detector

k) Recorders and integrators

Recorders are used to record the responses obtained from detectors after amplification, if necessary. They record the base line and all the peaks obtained, with respect time to time. Retention time for all the peaks can be found out from such recordings, but the area of individual peaks cannot be known.Integrators are improved variation of recorders with some data processing capabilities. They can be recorded the individual peaks with retention time, height and width of peaks, peak area, percentage of area, etc… integrators provide more information on peaks than recorders. Now a days the computers and printers are used for recording and processing the obtained data and for controlling several operations.

1.3 RP-UPLC

UPLC is a chromatographic technique that can separate a mixture of compounds, and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. Utilizing a small packaging particle sizes columns and ultra-high pack pressure UPLC can be regarded as a new invention for liquid chromatography. The major differences between HPLC and UPLC are the use of smaller particles in the column of the UPLC and the greater pressure at which the UPLC operates. Advantages of this UHPLC is decreases the length of column, reduces solvent consumption& saves time. Therefore by using smaller particles, speed and peak capacity (number of peaks resolved per unit time in gradient separations) can be extended to new limits. The technology takes full advantage of chromatographic principles to run separations Using columns packed with smaller particles (less than2.5µm) and/or higher flow rates.



Principle:

The underlying principle of UPLC is based on the van Deemter relationship which explains the correlation between flow rate and plate height[8]. The van Deemter equation (i) shows that the flow range with the smaller particles is much greater in comparison with larger particles for good results [9,10,11,12]

Van Deemter equation, whichis an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP or column efficiency).

$$H=A+B/v+Cv$$

Where;

A, B and C are constants

v is the linear velocity, the carrier gas flow rate.

*The A term is independent of velocity and represents "eddy" mixing. It is smallest when the packed column particles are small and uniform.

* The B term represents axial diffusion or the natural diffusion tendency of molecules. This effect is diminished at high flow rates and so this term is divided by v.

* The C term is due to kinetic resistance to equilibrium in the separation process.The kinetic resistance is the time lag involved in moving from the gas phase to the packing stationary phase and back again.

Efficiency is proportional to column length and inversely proportional to the particle size. Smaller particles provide increased efficiency as well as the ability to work at increased linear velocity without a loss of efficiency, providing both resolution and speed. Efficiency is the primary separation parameter behind UPLC since it relies on the same selectivity and retentivity as HPLC. In the fundamental resolution (Rs)

Chemistry of small particles: [13]

The design and development of sub-2 µm particles is a significant challenge, and researchers have been active in this area for some time to capitalize on their advantages. A commercially available non-porous, high iffiest small particle has poor loading capacity and retention due to low surface area. To maintain retention and capacity must use novel porous particles that can withstand high pressures. In 2000, hybride of silica & polymeric column was introduced which consist of classical sol-gel synthesis that incorporates carbon in the form of methyl groups, these columns are mechanically strong. They are highly efficient & can be operate at wide range of pH.But, in order to provide the kind of enhanced mechanical stability required for UPLC, a second generation bridged ethane hybrid (BEH) technology was developed. These 1.7 µm particles derive their enhanced mechanical stability by bridging the methyl groups in the silica matrix.

In order to provide the kind of enhanced mechanical stability required for UPLC, a second generation bridge dethane hybrid (BEH) technology was developed.

Instrumentation:

The UPLC System has been holistically designed to match the performance needs of innovative column chemistries with robust hardware, easy-to-use software and specialized support services. It consists of:

- Small, pressure-tolerant particles
- High-pressure fluidic modules
- Minimized system volume
- Negligible carryover
- Reduced cycle times
- Last response detectors
- Integrated system software and diagnostics

UHPLC instrument consists of following :

A. Sample Injection

B. UPLC Columns

C. Detectors

A. Sample Injection

In UPLC, sample introduction is critical. Conventional injection valves, either automated or manual, are not designed and hardened to work at extreme pressure. To protect the column from extreme pressure fluctuations, the injection process must be relatively pulse free and the swept volume of the device also needs to be minimal to reduce potential band spreading. A fast injection cycle time is needed to fully capitalize on the speed afforded by UPLC, which in turn requires a high sample capacity. Low volume injections with minimal carryover are also required to increase sensitivity. There are also direct injection approaches for biological samples.

B. UPLC Columns

Resolution is increased in a 1.7μm particle packed column because efficiency is better. Separation of the components of a sample requires a bonded phase that provides both retention and selectivity. Four bonded phases are available for UPLC separations:

(i) ACQUITY UPLCTM BEH C8 (straight chain alkyl columns),

(ii) ACQUITY UPLCTM BEH C18 (straight chain alkyl columns),

(iii) ACQUITY UPLC BEH Shield RP18 (embedded polar group column)

(iv) ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl),

(v) ACQUITY UPLC BEH Phenyl (phenyl group tethered to the silyl functionality with a C6 alkyl).

The binary solvent manager uses two individual serial flow pumps to deliver a parallel binary gradient. There are built-in solvent select valves to choose from up to four solvents. There is a 15,000-psi pressure limit (about 1000 bar) to take full advantage of the sub-2µm particles. The sample manager also incorporates several technology advancements. Using pressure assisted sample introduction, low dispersion is maintained through the injection process, and a series of pressures transducers facilitate self-monitoring and diagnostics. It uses needle-in-needle sampling for improved ruggedness and needle calibration sensor increases accuracy. Injection cycle time is 25 seconds without a wash and 60 sec with a dual wash used to further decrease carry over. A variety of micro titer plate formats (deep well, mid height, or vials) can also be accommodated in a thermostatically controlled environment. Using the optional sample organizer, the sample manager can inject from up to 22 micro titer plates. The sample manager also controls the column heater. Column temperatures up to 65°C can be attained.

C. Detectors

The detectors are use in UPLC analysis is UV/Visible detector. Detection of analytes is conventionally based on absorbance that is concentration sensitivity detectors. In UPLC the flow cell volume would have to be reduced to maintain concentration and signal. Based on Beer's Law, smaller volume conventional flow cells would also reduce the path length upon which the signal strength depends. A reduction in cross-section means the light path is reduced, and transmission drops with increasing noise. Therefore, if a conventional HPLC flow cell were used, UPLC sensitivity would be compromised. The ACQUITY Tunable UV/Visible detector cell consists of a light guided flow cell equivalent to an optical fiber. Light is efficiently transferred down the flow cell in an internal reflectance

mode that still maintains a 10mm flow cell path length with a volume of only 500mL. Tubing and connections in the system are efficiently routed to maintain low dispersion and to take advantage of leak detectors that interact with the software to alert the user to potential problems

1.4 METHOD DEVELOPMENT IN HPLC:

The steps involved in method development in HPLC are as follows:

1. Selection of HPLC method and initial system
2. Selection of optimum conditions
3. Selectivity optimization
4. System parameter optimization
5. Method validation

1. SELECTION OF THE HPLC METHOD AND INITIAL SYSTEM:

The choice of the HPLC method is based on the nature of the sample, namely, the molecular weight, polarity, ionic character and the solubility parameters, Type of Chromatography, Sample preparation, Column selection and column dimensions, Mobile phase preparation, Detectors.

2. SELECTION OF OPTIMUM CONDITIONS:

This step determines the optimum conditions to adequately retain all analytes. No analyte has a capacity factor of less than 0.5. No analyte has a capacity factor of greater than 10- 15 Determination of initial conditions. By performing 2 gradients runs differing in only the run time. Binarysystem based on either acetonitrile/water **or methanol/water should be used.**

3. SELECTIVITY OPTIMIZATION:

To achieve adequate selectivity mobile phase and stationary phase compositions are taken in account. To select these, the nature of analytes must be considered. Once the analyte types are identified, the relevant optimization parameters may be selected.

4. SYSTEM PARAMETER OPTIMIZATION:

This can be achieved by finding the desired balance between resolution and analysis time. Parameters involved include column dimensions, column packing, particle size, flow rate. Parameters may be changed without affecting capacity factor or selectivity

1.5 VALIDATION

Validation is the process of establishing documentary evidence demonstrating that a procedure, process, or activity carried out in testing and then production maintains the desired level of compliance at all stages.

In the pharmaceutical industry, it is very important that in addition to final testing and compliance of products, it is also assured that the process will consistently produce the expected results. The desired results are established in terms of specifications for outcome of the process.

Qualification of systems and equipment is therefore a part of the process of validation. Validation is a requirement of food, drug and pharmaceutical regulating agencies such as the US FDA and their good manufacturing practices guidelines.

Since a wide variety of procedures, processes, and activities need to be validated, the field of validation is divided into a number of subsections including the following:

- Equipment validation
- Facilities validation
- HVAC system validation
- Cleaning validation
- Process Validation
- Analytical method validation
- Computer system validation
- Packaging validation
- Cold chain validation

1.5.1 International conference on Harmonization (ICH)

The **International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use** (**ICH**) is a project that brings together the regulatory authorities of Europe, Japan and the United States and experts from the pharmaceutical industry in the three regions to discuss scientific and technical aspects of pharmaceutical product registration.

The purpose of ICH is to reduce or eliminate the need to duplicate the testing carried out during the research and development of new medicines by recommending ways to achieve greater harmonisation in the interpretation and application of technical guidelines and requirements for product registration. Harmonisation would lead to a more economical use of human, non-human animal and material resources, and the elimination of unnecessary delay in the global development and availability of new medicines while maintaining safeguards on quality, safety, and efficacy, and regulatory obligations to protect public health.

ICH Method Validation Parameters [18]

Table No: 2 ICH characteristics and guidelines.

Type of analytical procedure characteristics	IDENTIFICATION	TESTING FOR IMPURITIES quantitat.	limit	ASSAY - dissolution (measurement only) - content/potency
Accuracy	-	+	-	+
Precision				
Repeatability	-	+	-	+
Interm.Precision	-	+ (1)	-	+ (1)
Specificity (2)	+	+	+	+
Detection Limit	-	- (3)	+	-
Quantitation Limit	-	+	-	-
Linearity	-	+	-	+
Range	-	+	-	+

- signifies that this characteristic is not normally evaluated

+ signifies that this characteristic is normally evaluated

(1) In cases where reproducibility (see glossary) has been performed, intermediate precision is not needed

(2) Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s)

(3) May be needed in some cases

1.5.2 Method Validation Parameters

They have been defined in different working groups of national and international committees and are described in literature. The parameters as defined by the ICH and by other organization and authors are summarized below. They are

SYSTEM SUITABILITY /SYSTEM PRECISION:

Scope and Definition:

The System suitability/System Precision of an analytical procedure expresses the suitability of chromatographic conditions and an evident that the system is giving a precise value.

System suitability/System Precision is reported as percentage RSD of replicate injections, tailing factor of the major peak and Theoretical plates of standard solution.

System suitability/System Precision of the method was established by injecting five standard solutions continuously.

System Suitability Parameters:

RSD for 5 replicate injection of standard: NMT 2.0%

Tailing Factor : NMT 2.0

Theoretical plates : NLT 2000

SPECIFICITY (Placebo Interference):

Scope And Definition:

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present and also to provide an exact result which allows an accurate statement on the content or potency of the analyte in a sample.

Procedure:

The Specificity is given by the interference of placebo at the retention time of Teneligliptin.

Solutions of blank, placebo and Teneligliptinat nominal concentration were injected to assess the specificity of the analysis.

LINEARITY & RANGE:

Scope and Definition:

The ICH defines the linearity of an analytical procedure as the ability (within a given range) to obtain test result of variable data which are directly proportional to the concentration (amount of analyte). The data variables that can be used for quantitation of the analyte are the peak areas, Peak heights or the ratio of peak areas (Heights) of analyte to internal standard peak. Quantitation of the analyte depends on it obeying Beer's law and is linear over a concentration range.

Linearity is demonstrated directly by dilution of standard stock solution. For the establishment of linearity, 5 concentrations Teneligliptin were used.

Calculation of Correlation Coefficient:

Correlation(r) = $N\Sigma XY - (\Sigma X)(\Sigma Y) / \text{Sqrt}([N\Sigma X^2 - (\Sigma X)^2][N\Sigma Y^2 - (\Sigma Y)^2])$

N = Number of value

X = First Score

Y = Second Score

ΣXY = Sum of the product of first and Second Scores

ΣX = Sum of First Scores

ΣY = Sum of Second Scores

ΣX^2 = Sum of square First Scores

ΣY^2 = Sum of square Second Scores

Slope: Calculation:

$y = mx + c$

Where, x and y are point of the line.

m is the Slope ; C is the y intercept

PRECISION:

METHOD PRECISION:

Scope and Definition:

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from the multiple samples of the same homogeneous sample under prescribed conditions.

Repeatability: Repeatability is a measure of the precision under the same operating conditions over a short interval of time. It is sometimes referred to as intra assay precision. Repeatability is assessed using three concentration levels at low, middle and high level of working concentration of Teneligliptin. Three determinations are studied at each level and the sample is prepared independently and analyzed. The standard deviation and related standard deviation (Co-efficient of variation) for the assay percentage recovery value are reported.

Formulae used for Calculations:

Average:

$$X_A = \frac{[X_1 + X_2 + X_3 + X_4 + \ldots\ldots\ldots\ldots\ldots + X_N]}{N}$$

Where,

X_A - Average

X – Function

N – Total number of function (Here N = 6)

Standard Deviation:

$$\text{Standard Deviation} = \frac{[X_1 - X_A]^2 + [X_2 - X_A] + \ldots\ldots\ldots + [X_N - X_A]^{21/2}}{N - 1}$$

Where,

X – Function

N – Number of function

X_A - Average

Related Standard Deviation:

$$RSD = \frac{\text{Standard Deviation X 100}}{\text{Average}}$$

INTERMEDIATE PRECISION:

Scope and Definition:

Intermediate precision is defined as the variation within the same laboratories with respect to the change in the user (Chemist). The extent to which intermediate precision needs to be established depends on the circumstances under which the procedure is intended to be used. Typical parameters that are investigated include day-to-day variation, analyst variation, and equipment variation. Depending on the extent of the study, the use of experimental design is encouraged. Experimental design will minimize the number of experiments that need to be performed. The final study is reported of standard deviation, relative standard deviation (coefficient of variation).

Procedure:

The parameter for the intermediate precision considered here is the variability on different days in terms of the same method. Outcome of data is being grouped separately Day 1 and Day 2 below to yield to statistical inference.

Prepared six samples as above and inject separately and standard deviation, relative standard deviation (coefficient of variation).

Calculation:

$$= \frac{\text{Sample area x Standard dilution x purity X average net content X 100}}{\text{Standard area X Sample dilution X 100 X label claim}} \text{ X } 0.6829$$

ACCURACY:

Scope and Definition:

The accuracy of an analytical procedure express the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.

Accuracy is reported as percentage assay of the known added amount of analyte in the sample.

Procedure:

Accuracy of the method was established by application of the analytical procedure to synthetic mixtures of the drug components (placebo) to which known amounts of analyte was added and studied at three concentration levels at low, middle and high level of working concentration of Teneligliptin. Three determinations are studied at each level and the sample is prepared independently and analyzed

ROBUSTNESS:

Scope and Definition:

The evaluation of robustness should be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure.

In the case of liquid chromatography, examples of typical variations are:

- Influence of variations of pH in a mobile phase;
- Influence of variations in mobile phase composition;
- Different columns (different lots and/or suppliers);
- Temperature;
- Flow rate.

Depending on the extent of the study, the use of experimental design is encouraged. Experimental design will minimize the number of experiments that need to be performed. The final study is reported of standard deviation, relative standard deviation (coefficient of variation).

Procedure:

The parameter for the Robustness study considered here is the deliberate but slight change in pH of the buffer used in the mobile phase and Flow rate. Outcome of data is being grouped separately to yield to statistical inference. The percentage RSD of assay for the results obtained with Method Precision/Accuracy and the Robustness data is being grouped separately to yield to statistical inference.

DETECTION LIMIT:

Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

Based on Visual Evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods. The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

Based on Signal-to-Noise

This approach can only be applied to analytical procedures which exhibit baseline noise.

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit.

Based on the Standard Deviation of the Response and the Slope

The detection limit (DL) may be expressed as:

$$DL = 3.3\ \sigma / S$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways,

For example:

Based on the Standard Deviation of the Blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

Based on the Calibration Curve

A specific calibration curve should be studied using samples containing an analyte in the range of DL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

The detection limit and the method used for determining the detection limit should be presented. If DL is determined based on visual evaluation or based on signal to noise ratio, the presentation of the relevant chromatograms is considered acceptable for justification.

Several approaches for determining the detection limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

QUANTITATION LIMIT

Several approaches for determining the quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

Based on Visual Evaluation: Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods. The quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and

by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

Based on Signal-to-Noise Approach: This approach can only be applied to analytical procedures that exhibit baseline noise. Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal-to-noise ratio is 10:1.

Based on the Standard Deviation of the Response and the Slope

The quantitation limit (QL) may be expressed as:

$$QL = 10\ \sigma\ /S$$

Where,

σ = the standard deviation of the response

S = the slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte. The estimate of σ may be carried out in a variety of ways

for example:

Based on Standard Deviation of the Blank

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

Based on the Calibration Curve: A specific calibration curve should be studied using samples, containing an analyte in the range of QL. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

The quantitation limit and the method used for determining the quantitation limit should be presented. The limit should be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the quantitation limit.

LITERATURE REVIEW

Sohan S. Chitlange[31] ***et al,*** has been developed a simple, accurate, precise and economical HPLC and UV method has been developed and validated for the estimation of teneligliptin hydrobromide hydrate (THH) in bulk and tablet dosage form. Isocratic elution at the flow rate of 1.0 ml/min was employed on a Kromasil 100-5-C8 column at ambient temperature. The mobile phase consisted of Methanol: 0.025M phosphate buffer pH adjusted to 3 with o-phosphoric acid (60:40 v/v). The detection wavelength was at 254nm. Linearity was observed in the concentration range of 10-100 µg/ml. The retention time for Teneligliptin was 4.14 min. In stability testing, teneligliptin was found susceptible to alkali hydrolysis and oxidatative degradation. Because the method could effectively separate the drug from its degradation products, it can be used as a stability indicating method. First order derivative UV spectrophotometric method was also developed using methanol as solvent at analytical λ 261.0 nm. Beer's law was obeyed in the concentration range of 5-50 µg/ml and r2 =0.9996. The proposed methods were validated according to the ICH guidelines. Both the developed methods are accurate and precise and can be used for routine quality control analysis of Teneligliptin in bulk and pharmaceutical formulation. In case of HPLC as well resolved peak is obtained for Teneligliptin after degradation, method is also suitable for stability studies.

Vinutha Kommineni [32]***et al,*** has been developed A new stability indicating RP HPLC method has been developed and validated for simultaneous estimation of Metformin Hydrochloride and Teneligliptin in bulk and dosage forms. The method involves separation on YMC C18column (150mm x 4.6mm x5µm particle size). The optimized mobile phase consists of Phosphate buffer (pH 3) and Acetonitrile (80:20v/v) with a flow rate of 0.8ml/min and UV detection at 220mn. Retention time was 2.138min (Metformin Hydrochloride), 2.943min (Teneligliptin), 5.075 Pioglitazone. Linearity range was 9.98-600ug/ml (Metformin Hydrochloride), 0.51-24ug/ml (Teneligliptin). Accuracy was in the range of 99.41-100.74% for both drugs. Precision was 0.8% and 0.9% for Metformin Hydrochloride and Teneligliptin. LOD and LOQ are 0.72ug/ml and 2.40ug/ml for Metformin Hydrochloride, 0.15ug/ml and 0.51ug/ml for Teneligliptin. The method developed is sensitive, accurate and precise. Retention time and run time were also less

and hence the method is economical. When applied for tablet assay, drug content was within 99.89-100.74 % of labeled content. Forced degradation studies indicated the suitability of the method for stability studies.

Shailesh V. Luhar[33] *et al,* worked on Simultaneous Estimation of Teneligliptin Hydrobromide Hydrate and its Degradation Product by RP-HPLC Method. A simple, rapid, precise and accurate reversed-phase stability-indicating RP-HPLC method was developed and validated for the simultaneous determination of Teneligliptin hydrobromide hydrate in marketed formulation (tablets). The method has shown adequate separation for Teneligliptin hydrobromide from its associated main impurities and their degradation products. Separation was achieved on a Shisedo C18column, 5µm, 250mm × 4.6 mm i.e. column using a mobile phase consisting of Acetonitrile:Methanol: Water (30:40: 30 % v/v/v) at a flow rate of 1.0ml/min and UV detection at 246nm. The drugs are subjected to acid hydrolysis, alkaline hydrolysis, oxidative degradation and thermal degradation to apply force degradation testing. The linearity of the proposed method was investigated in the range of 50-300µg/ml (r2= 0.9996). The limit of detection was2.78 µg/ml and the limit of quantification was 8.45 µg/ml

Atul T. Hemke[34] *etal*, reported work on HPLC and UV-spectrophotometric estimation of teneligliptin from tablet dosage form. The present work proposed precise, accurate and validated HPLC and UV-spectrophotometric methods for estimation of Teneligliptin from its tablet dosage form. The UV-spectrophotometric estimation includes Calibration curve, Area under curve (AUC) and First order derivative method based on measurement of absorbance at a selected wavelengths using UV-visible spectrophotometer with 1cm matched quartz cell and distilled water as a solvent. All UV-spectrophotometric methods obeyed Beer's-lambert's law in the concentration range of 10-70µg/mL, with correlation coefficient value less than 1. The chromatographic separation was achieved by isocratic mode with a mixture of methanol: phosphate buffer (pH 7.2) in the ratio of 70:30 v/v as the mobile phase using Shodex C18 column as stationary phase at flow rate of 1mL/min and detection wavelength of 244nm. The retention time was found to be 5.753min. The percent amount of drug estimated by all developed methods was nearly 100%, found to

be in good agreement with label claim of marketed tablet formulation. The recovery study was carried out at five different levels and results were found to be satisfactory. The validation parameters like accuracy, precision, ruggedness, linearity and range were studied for all the developed methods and were found to be within limits. Stress testing under various conditions such as pH (acid/base), temperature, light, oxidation, humidity was carried out and % undegraded drug was calculated

T. N. V. Ganesh Kumar[35] *et al,* worked on Method development, validation, and stability studies of teneligliptin by RP-HPLC and identification of degradation products by UPLC tandem mass spectroscopy. Background: Teneligliptin is a new FDA approved drug for treating Diabetes Mellitus. There are no reported evidences for their identified degradation products and their effects on humans. Methods: A simple and new stability indicating RP-HPLC method was developed and validated for identification of Teneligliptin and its degradants on Kromasil 100- 5C18 (250×4.6mm, 5µm) column using pH 6.0 phosphate buffer and acetonitrile (60:40 v/v) as a mobile phase in isocratic mode of elution at a flow rate of 1.0 mL/min. The column effluents were monitored by a variable wavelength UV detector at 246 nm. The method was validated as per ICH guidelines. Forced degradation studies of Teneligliptin were carried out under acidic, basic, neutral (peroxide), photo and thermal conditions for 48 hours at room temperature. The degradation products were identified by HPLC and characterized by UPLC with tandem mass spectroscopy (LC/MS/MS). Results: UPLC MS/MS data shown major peaks, observed at 375.72, 354.30, 310.30, 214.19, 155.65, 138.08 and 136.18 m/z. Conclusion: Degradation was observed in base, peroxide and thermal stressed samples, but not in acid and photolytic stressed samples.

M.Chandana[36] *et al,* worked on analytical method development and validation of teniligliptin in phrmaceutical dosage form by RP-HPLC method. A reverse phase liquid chromatography (RP-HPLC) method has been developed and subsequently validated for the determination of Teneligliptin in Bulk and its pharmaceutical formulation. Separation was achieved with a Zodiac C18 column(250×4.6 mm I.D., 5µm particle size , methanol: acetonitrile (90:10) v/v as mobile phase at a flow rate of 1.0 ml/min , pH 5.2 adjusted

with 0.10% orthophosphoric acid and the Column temperature was maintained at 25°C. UV detection was performed at 235 nm and sample temperature was maintained at 5°C with a run time of 10min . The method is simple, rapid, and selective. The described method of Teneligliptin is linear over a range of 25 to 150µg/ml with correlation coefficient of 0.9991 respectively .The method precision for the determination of assay was below 2.0% RSD. The method enables accurate, precise, and rapid analysis of Teneligliptin . It can be conveniently adopted for routine quality control analysis of Bulk and pharmaceutical formulations.

Vishnu C. Shinde[37] ***et al,*** worked on Development and validation of UV spectrophotometric method and high performance thin layer chromatographic (HPTLC) method for estimation of teneligliptin hydrobromide in pharmaceutical preparation . Simple, rapid, sensitive, precise and specific UV spectrophotometric and High-performance thin layer chromatographic (HPTLC) methods for the determination of Teneligliptin Hydrobromide both in bulk drug and pharmaceutical dosage form were developed and validated. In UV spectrophotometric method, the solutions of Teneligliptin HBr were prepared in water. The standard solution of Teneligliptin HBr showed maximum absorption at wavelength 243.5 nm. The drug obeyed Beer–Lambert's law in the concentration range of 10– 90 µg/mL with coefficient of correlation (r2) of 0.999. For HPTLC method, the method employed aluminium plates precoated with silica gel G60 F254 as the stationary phase. The solvent system consisted of toluene: chloroform: ethanol: diethyl amine in the proportion of 4:4:1:1, v/v/v/v. This solvent system was found to give compact spots for Teneligliptin HBr with Rf value 0.16 ± 0.01. Densitometric analysis of Teneligliptin HBr was carried out in the absorbance mode at 254 nm. Linear regression analysis showed good linearity (r2 =0.998) with respect to peak area in the concentration range of 100–600 ng/spot. The developed methods were validated as per the ICH guidelines. Statistical analysis proved that the methods are repeatable and specific for the estimation of the said drug. These methods can be adopted in routine assay analysis of Teneligliptin HBr in bulk or tablet dosage form.

Raja Haranadha Babu Chunduri [38] *et al,* worked development and validation of LC-MS/MS method for quantification of teneligliptin in human plasma and its application to a pharmacokinetic study. A selective, sensitive and rapid liquid chromatographic method with electro spray ionization tandem mass spectrometric detection has been developed and validated for quantification of teneligliptin in human plasma using repaglinide as internal standard (IS). The analytes and IS were extracted from 50 µL of plasma by liquid-liquid extraction technique using methyl tert - butyl ether which offers high sensitivity, wide linearity without interferences form endogenous matrix components. In a short chromatographic run of 1.50 min run time, separation was achieved on a Hypersil Gold C18 column using a mobile phase composed of 0.1% formic acid in Milli-Q water/0.1% formic acid in acetonitrile in gradient elution mode. The quantification of teneligliptin was performed in a positive electro spray ionization mode and multiple reaction monitoring (MRM). Response was a linear function of concentration in the ranges of 0.1 to 4000 ng/mL for teneligliptin with r2> 0.99. The intra- and inter-day precision and accuracy results were below 15% and acceptable as per Food and Drug Administration (FDA) guidelines. Stability of teneligliptin was established in a battery of stability studies, i.e. bench top, auto sampler and long term storage stability as well as freeze thaw cycles. The validated method can be used as a routine method to support pharmacokinetic studies.

Ruchi P Pandya[39] *et al,* worked on development and validation of RP-HPLC method for simultaneous Estimation of teneligliptin hemipentahydrobromide hydrate and
Metformin hydrochloride in their combined tablet dosage form. A simple, rapid and precise reverse phase high performance liquid chromatographic method has been developed for simultaneous estimation of Teneligliptin hemipentahydrobromide hydrate and Metformin Hydrochloride in their combine tablet dosage form. Chromatography was performed on a Phenomenex luna ODS C18 (250mm X 4.6 mm) 5□m column using 0.025M Dipotassium hydrogen phosphate buffer:Acetonitrile (75:25 v/v) as a mobile phase. The detection was carried out at 238 nm with a flow rate of 1.0 mL/min. The retention times were 2.167 and 6.033 minutes for Metformin Hydrochloride and Teneligliptin hemipentahydrobromide hydrate, respectively. Proposed method was

validated as per ICH guidelines for linearity, accuracy, precision, specificity and robustness for estimation of Teneligliptin hemipenta hydrobromide hydrate and Metformin Hydrochloride in their combined Tablet dosage form and results were found to be satisfactory. The linearity of the method was excellent over a concentration range 5 to 15 µg/mL for Teneligliptin hemipenta hydr bromide hydrate and 125 to 375 µg/mL for Metformin Hydrochloride. The correlation coefficient was 0.997 and 0.999 for Teneligliptin hemipenta hydrobromide hydrate and Metformin Hydrochloride, respectively. The limit of detection was 0.771 µg/mL and 2.959 µg/mL for Teneligliptin hemipenta hydrobromide hydrate and Metformin Hydrochloride, respectively. The limit of quantitation was 2.336 µg/mL and 8.967 µg/mL for Teneligliptin hemipenta hydrobromide hydrate and Metformin Hydrochloride, respectively. The relative standard deviation values for repeatability, intraday precision and interday precision studies were less than 2 % and % recovery was between 98 % to 102 % for both drugs. So the proposed method was found to be suitable as per ICH Guidelines Q2(R1) for the routine estimation of Teneligliptin hemipentahydrobromide hydrate and Metformin Hydrochloride in their Combined Tablet dosage form.

DRUG PROFILE

TENELIGLIPTIN[19-29]:

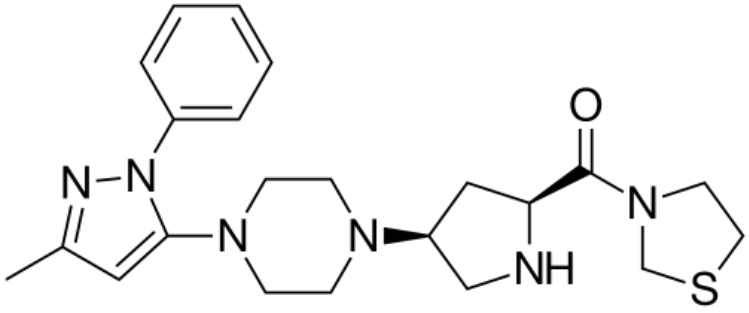
Teneligliptin is a pharmaceutical drug for the treatment of type 2 diabetes mellitus. It belongs to the class of anti-diabetic drugs known as dipeptidyl peptidase-4 inhibitors or "gliptins". Teneligliptin HBr hydrate is a novel, potent, peptidomimetic, and long-acting DPP-4 inhibitor which got approval for the treatment of T2DM in Japan and Argentina (2012), Korea (2014) and India (2015) Teneligliptin drug inhibit the enzyme dipeptidyl peptidase-4 which degrades incretin, a hormone adjusting blood glucose level.

Brand name: Tenelia

Chemical IUPAC Name: {(2*S*,4*S*)-4-[4-(3-Methyl-1-phenyl-1*H*-pyrazol-5-yl)-1 - piperazinyl]-2-pyrrolidinyl}(1,3-thiazolidin-3-yl)methanone

Molecular formula: $C_{22}H_{30}N_6OS$

Chemical Structure:



Molecular formula : $C_{22}H_{30}N_6OS$

Molecular weight : 426.58

Description : White powder

Boiling Point : 661.189°C at 760 mmHg

Melting point : 202 °C

Solubility : 10 mM in DMSO and in methanol

Category **:** Anti-diabetic

Metabolite **:** inactive Metabolites.

Bioavailability **:** 63-85 %

Protein Binding **:** 78-80 %

Elimination

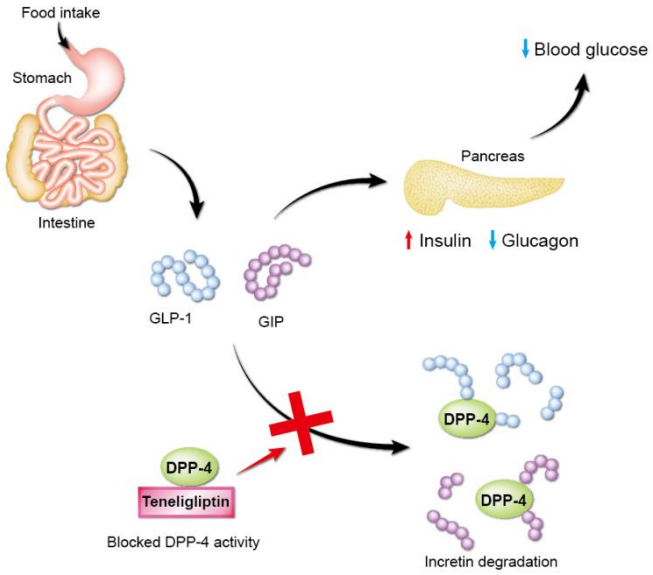
Clearance **: Feaces (46.5 %) and urine (45.4 %)**

$T_{1/2}$ **:** 26.9 hours

CLINICAL PHARMACOLOGY:

Mechanism of Action: Teneligliptin inhibited human dipeptidyl peptidase-4 (DPP-4) enzyme activity with the IC50 =1nM, more than 150 fold selectivity against DPP-8 and DPP-9 which suggested little off-target skin lesion side effect. By DPP-4 inhibtion,

Teneligliptin prevented the degradation of incretins GLP-1, GIP and promoted insulin release which prevented blood glucose increase after food intake with little hypoglycemia risk during lifetime taken.



Pharmacokinetics

Teneligliptin is rapidly absorbed in healthy volunteers after a single radiolabeled 20 mg dose, with maximum plasma concentrations attained in 1.33 hr. The drug is 78% - 80% bound to plasma proteins. An overview of Teneligliptin pharmacokinetics is mentioned in Table 3. In humans, Teneligliptin is primarily metabolized by cytochrome P450 (CYP) 3A4 & flavin monooxygenases (FMO) 1 and 3 to several metabolites of unknown biological activity. In vitro, Teneligliptin is a weak inhibitor of CYP2D6, CYP3A4 and FMO, but shows no inhibitory effect on CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C8/9, CYP2C19 and CYP2E1. Teneligliptin does not induce CYP3A4 or CYP1A2 There were no clinically relevant drug-drug interactions when Teneligliptin was co-administered with Ketoconazole (a potent CYP3A4 and P-glycoprotein inhibitor) Metformin or Canagliflozin in healthy volunteers. No clinically relevant effects on the pharmacokinetics of Teneligliptin were observed when it was coadministered with Glimepiride or Pioglitazone. Teneligliptin follows dual mode of excretion i.e. renal & hepatic. At least 90% of the radiolabeled dose of Teneligliptin was excreted within 216 h, with 45.4% excreted in the urine and 46.5% excreted in the faeces Approximately 21%

of Teneligliptin is excreted in the urine as unchanged drug. Teneligliptin has long half-life of 26.9 hours which offers convenient once a day administration

CLINICAL STUDIES:

Criteria

Inclusion Criteria:

- Patients who has been receiving a stable dose and regimen of insulin over 12 weeks before administration of investigational drug
- Patients who are under dietary management and taking therapeutic exercise for diabetes over 12 weeks before administration of investigational drug
- Patients whose HbA1c is between 7.5% and 10.5%
- Patients who were not administered diabetes therapeutic drugs prohibited for concomitant use within 12 weeks before administration of investigational drug.

Exclusion Criteria:

- Patients with type 1 diabetes, diabetes mellitus caused by pancreas impairment, or secondary diabetes (Cushing disease, acromegaly, etc)
- Patients who are accepting treatments of arrhythmias
- Patients with serious diabetic complications
- Patients who are the excessive alcohol addicts
- Patients with severe hepatic disorder or severe renal disorder.
- Patients who are pregnant, lactating, and probably pregnant patients, and patients who cannot agree to contraception

Indications and usage:

Teneligliptin is used as a monotherapy, in addition to diet and exercise, to reduce high blood sugar levels in patients with type 2 diabetes mellitus.

Contradictions:

- To any allergic reactions (itch, rash, etc.) to any medicines, ketosis, diabetic coma or precoma stage, type 1 diabetes mellitus, infection, in pre/post operative period, trauma.
- In pregnant conditions or breastfeeding.
- If you are taking any other medicinal products. (Some medicines may interact to enhance or diminish medicinal effects. Beware of over-the-counter medicines and dietary supplements as well as other prescription medicines.)

Dose:

- In general, for adults, take 1 tablet (20 mg of teneligliptin) at a time, once a day. If the effect is insufficient, the dose may be increased up to 2 tablets (40 mg) at a time, once a day. Strictly follow the instructions.
- If you miss a dose, take a dose as soon as possible. If it is almost time for the next dose, skip the missed dose and continue your regular dosing schedule. You should never take two doses at one time.

AIM & OBJECTIVE OF PRESENT WORK

Teneligliptin is a pharmaceutical drug for the treatment of type 2 diabetes mellitus. It belongs to the class of anti-diabetic drugs known as dipeptidyl peptidase-4 inhibitors or "gliptins". Teneligliptin HBr hydrate is a novel, potent, peptidomimetic, and long-acting DPP-4 inhibitor which got approval for the treatment of T2DM in Japan and Argentina (2012), Korea (2014) and India (2015) Teneligliptin drug inhibit the enzyme dipeptidyl peptidase-4 which degrades incretin, a hormone adjusting blood glucose level.

Till date only few analytical methods in RP- HPLC have been reported for the determination of Teneligliptin in pharmaceutical dosage forms. Existing literature reveals that analytical methods like HPLC,UV and HPTLC methods were available but no UHPLC Methods were reported. Therefore, the present study has been undertaken in order to develop a new, simple, rapid, efficient and reproducible RP-UHPLC method for the analysis of Teneligliptin tablets.

Materials used:

- **1-Octane sulfonic acid Sodium salt**
  HPLC Grade
  Maker: RANKEM
- **Acetonitrile**
  HPLC Grade
  Maker: RANKEM
- **Ortho-Phosphoric Acid**
  AR Grade
  Maker: RANKEM
- **Methanol**
  HPLC Grade
- **HPLC Grade Water**

METHOD USED FOR ASSAY OF TENELIGLIPTIN TABLETS

STRENGTH: 20 mg

Chromatographic parameters:

Column : Phenomenex Luna C18, 50×3.0mm, 3µm particle size

Flow rate : 0.5 mL/min

Run Time : 3 minutes

Wavelength : 245nm

Temperature : Ambient

Injection Volume : 2µL

Mobile phase composition: pH 3.5 Buffer: Acetonitrile (50:50v/v)

Preparation of pH 3.5 Buffer solution: Weigh and dissolve 1.00 g of 1-Octane sulfonic acid Sodium salt to 1000 ml of water. Adjust pH of solution to 3.5with dilute orthophosphoric acid.

Preparation of mobile phase:

1. Mix pH 3.5 Buffer solution and acetonitrile in the ratio 50:50(v/v)
2. Filter through 0.45µm membrane filter and degas for about 10min.

Note: Do not use the mobile phase preparation beyond 2days of bench top or 5days in refrigerator.

Diluent: Use mobile phase as diluent.

Preparation of standard stock solution:

Weigh accurately and transfer about 75 mg Teneligliptin Hydrobromide working standard which is equivalent to about 50 mg of Teneligliptin into a 100ml volumetric flask, add about 35ml of diluent, sonicate to dissolve the material completely, dilute to volume with diluent and mix.

Preparation of standard solution:

1. Pipette 5ml of the above solution into a 25ml volumetric flask, dilute to volume with diluent and mix.
2. Filter through 0.45µm filter.

Note: Do not use the standard preparation beyond 2days in refrigerator.

Test preparation:

1. Weigh and crush not less than 20 tablets using mortar and pestle.
2. Weigh and transfer the tablet powder equivalent to about 10 mg of Teneligliptin into a 100ml volumetric flask, add about 75ml of diluent, sonicate for 30minutes with intermediate shaking and dilute to volume with diluent.
3. Centrifuge a portion of above solution at 2500rpm for about 10minutes by using centrifuge tubes with caps.

4. From the above supernatant solution is used.
5. Filter through 0.45µm filter.
6. **Note:** Do not use the test preparation beyond 2days on bench top or 5days in refrigerator.

System suitability:

1. Inject about 10 µL portion of standard solution into the chromatographic system and measure the response of major peak.
2. The tailing factor for Teneligliptin peak should be NMT 2.0
3. The RSD for the area of Teneligliptin peak obtained from the 5 replicates injections of standard preparation should be NMT 2.0%.

Procedure:

Inject about 10 µL portion of diluent and test preparation into the chromatograph, record the chromatogram and measure the response of major peak.

Calculations:

$$\frac{\text{Sample area x Standard dilution x purity x average weight x 100 X 0.6829}}{\text{Standard area x Sample dilution x 100 x label claim}}$$

Where: 0.6829 is conversion factor

ASSAY METHOD DEVELOPMENT AND OPTIMISATION

1. Detection method & Selection of wavelength:

Known concentration of Teneligliptin working standard was taken and dissolved in solvent such that the standard solution contains about 10ppm. Placebo and blank solutions were also prepared. All these solutions were scanned between 200 to 400nm using UV-Visible spectrophotometer.From the UV spectrum at the drug shows good absorbance 245 nm.

After reviewing the chromatograms and peak purity chromatograms a wavelength of 245 is selected as the optimum wavelength for this drug.

2. Selection of column:

As the Method is UHPLC various columns cannot be selected as the method is highly accurate, hence a particular UHPLC column is selected i.e., Phenomenex Luna C18(2) ; 3µm ; (3*50mm) which gave good peak shape, retention time, tailing factor,column efficiency. Hence Phenomenex column is selected

3. Optimization of mobile phase:

On the basis of retention propperty study results of the drug and reviewing the results“1-Octane sulfonic acid Sodium salt” is decided as the buffer preparation to be used.

Selection of pH of the buffer:

‘pH’ plays very an important role in achieving the chromatographic separation as it controls the elution properties by controlling ionizationcharacteristics.

By altering the pH of the mobile phase different trials were carried out and finally optimized pH was found to be 3.5

At pH 3.5 peak shape, peak tailing and theoretical plate count was found to be satisfactory and hence 3.5 is decided as the pH of the Buffer.

Mobile phase composition:

Many trials on composition of Buffer and Acetonitrile were made to decide the ultimate composition of the mobile phase.

After reviewing many trials good peak shape, retention time, tailing factor, theoretical plates are obtained with the mobile phase composition Buffer: ACN (50:50). Hence it was finalized.

4. Selection of flow rate:

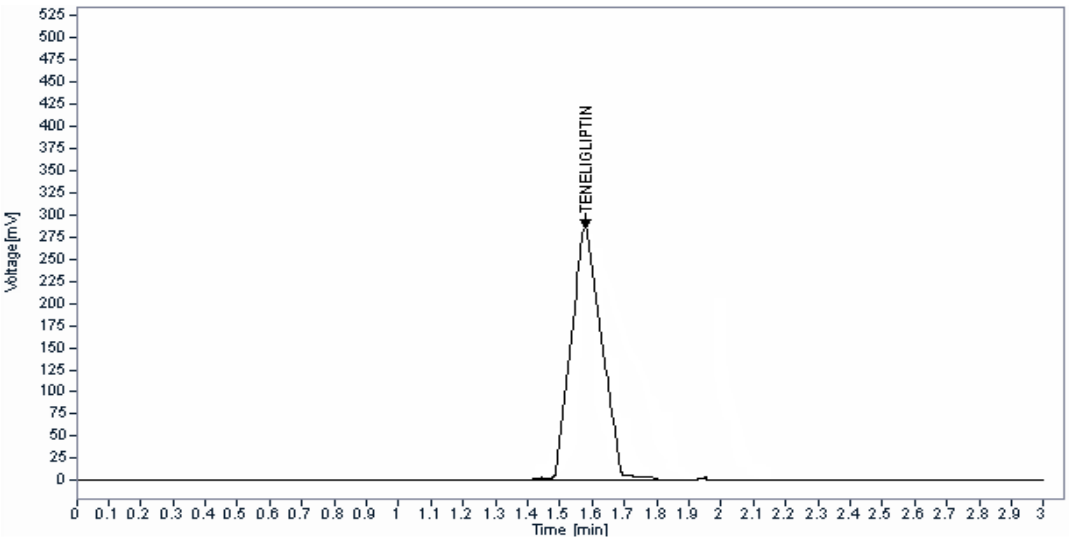
Flow rate selection is done depending on the retention time, peak symmetry, hence many trials were made to decide the flow rate and 0.5 ml /min was found to be acceptable as the peak is sharp. Hence 0.5 ml/ min flow rate was selected for this project

METHOD DEVELOPMENT PHASE CHROMATOGRAMS:

TRIAL I:

Chromatographic Conditions:

Mobile phase	:	Water : Methanol (80 :20% v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl

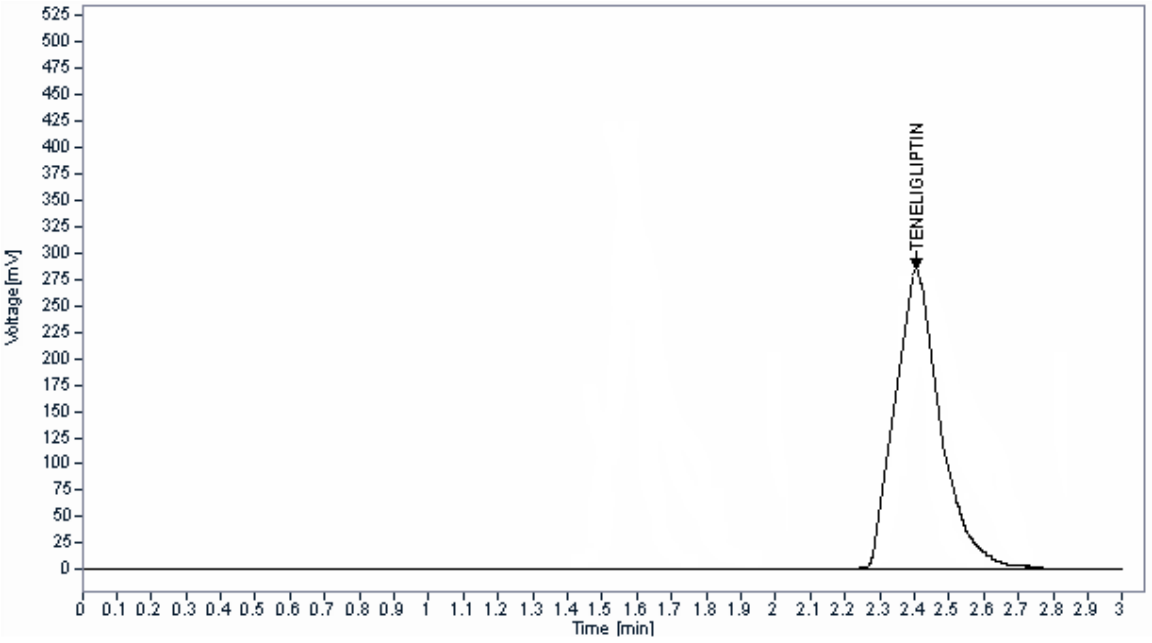


Observation: From the above chromatogram it was observed that the peak is broad and shows tailing.

TRIAL II:

Chromatographic Conditions:

Mobile phase	:	Water : ACN (70:30 % v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl

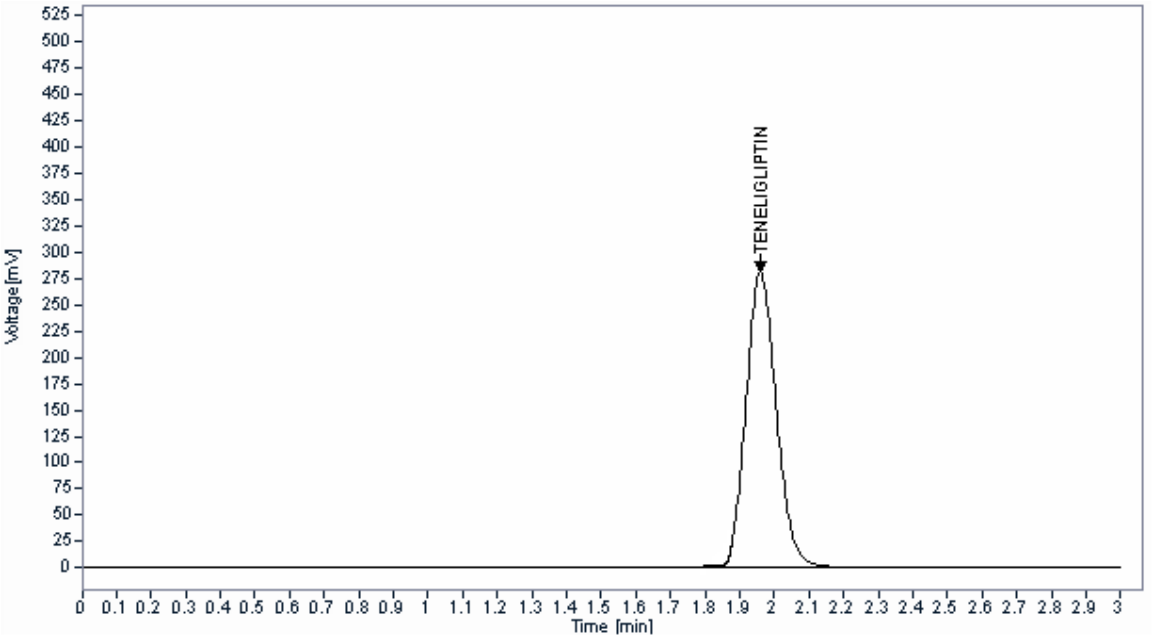


Observation: From the above chromatogram it was observed that the peak shape and tailing factor is not upto the aim of the project

TRIAL III:

Chromatographic Conditions:

Mobile phase	:	ACN: Phosphate buffer pH 6.0 (30:70% v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl

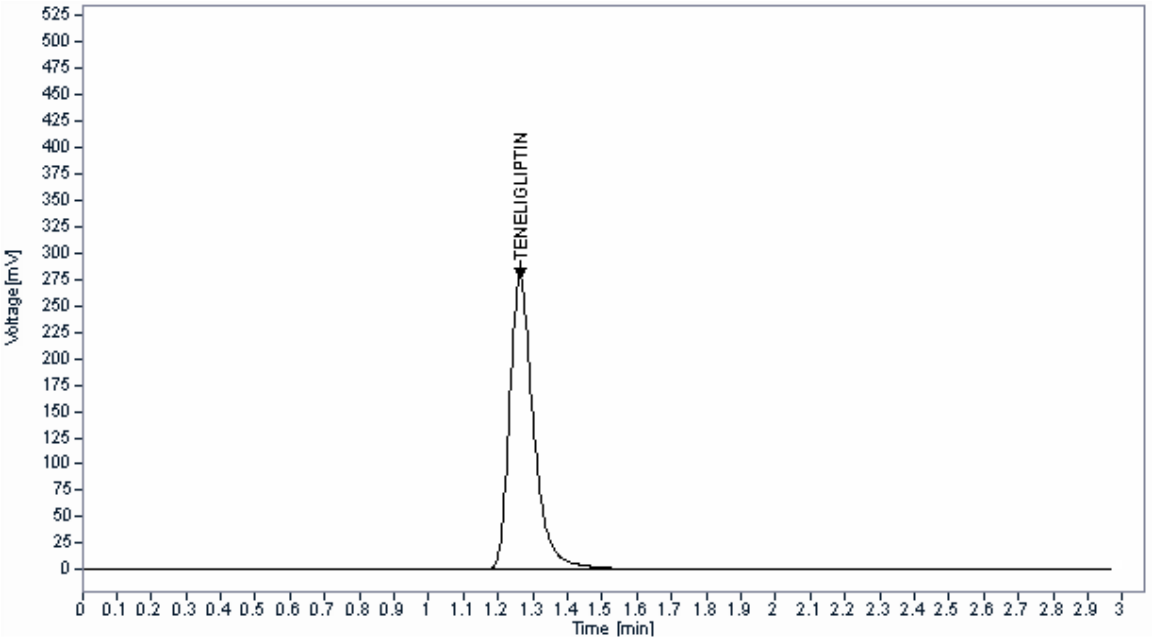


Observation: From the above chromatogram it was observed that the peak shape is not sharp and retention time is not upto the aim of the project.

TRIAL IV:

Chromatographic Conditions:

Mobile phase	:	ACN: Phosphate buffer pH 4.5 (40 :60 % v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl

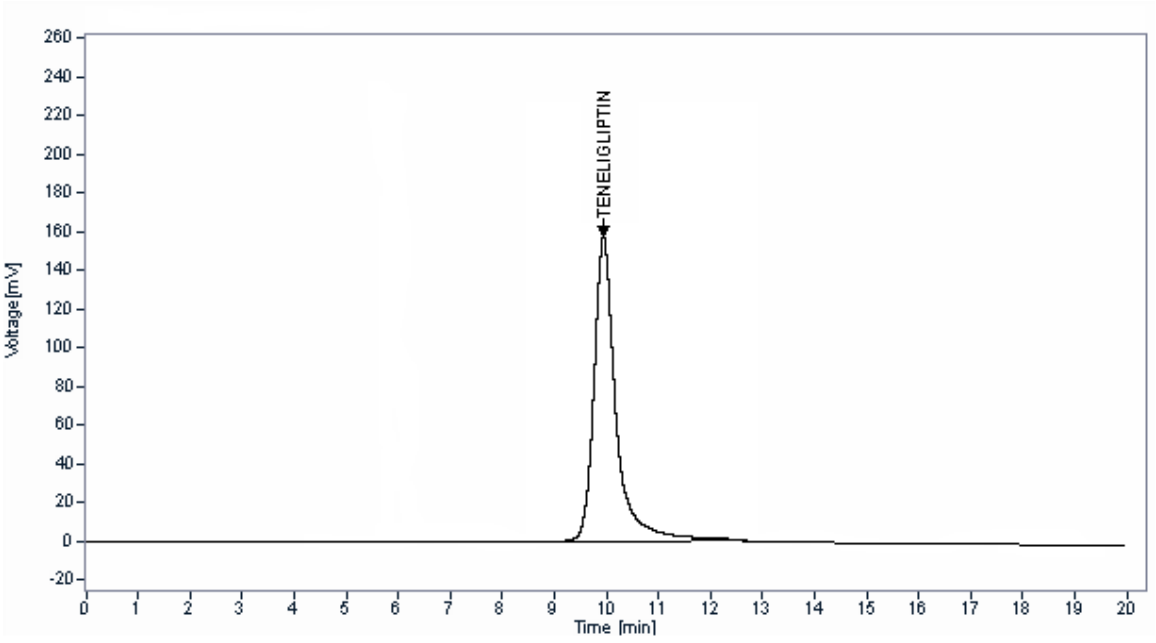


Observation: From the above chromatogram it was observed that the peak shape is not sharp and retention time is not upto the aim of the project

TRIAL V:

Chromatographic Conditions:

Mobile phase	:	Methanol: Phosphate buffer pH 4.0 (50:50 % v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl

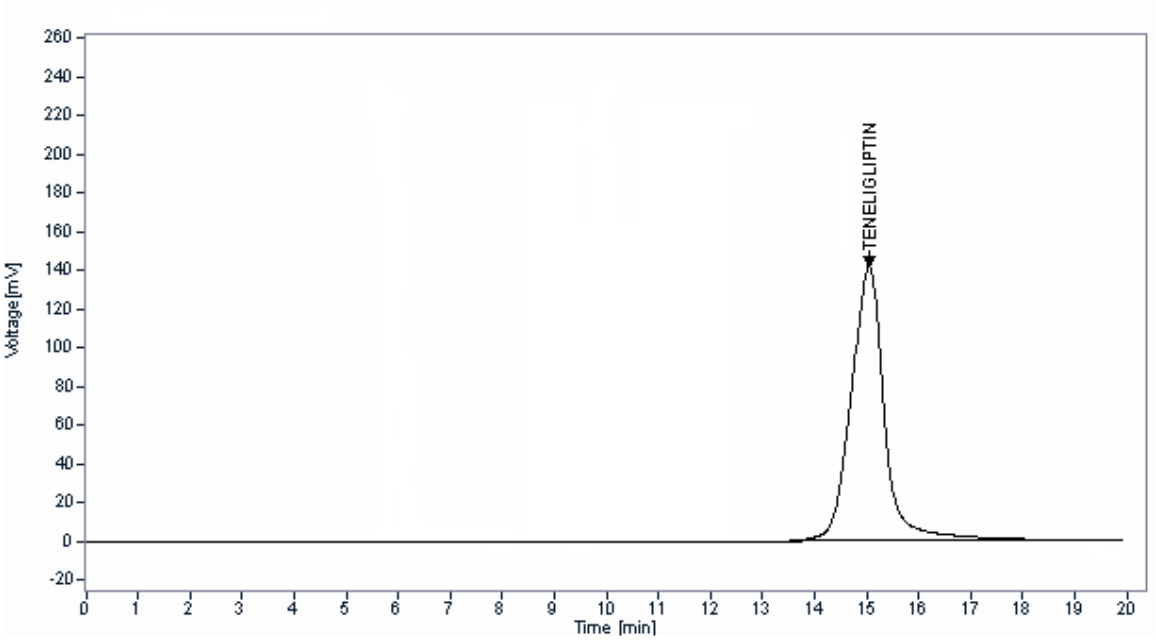


Observation: From the above chromatogram it was observed that theobtained Retention time, peak shape, tailing factor, theoretical plates is not upto the aim of the project

TRIAL VI:

Chromatographic Conditions:

Mobile phase	:	ACN: 1-Hexane sulfonic acid Sodium salt pH 3.5(50:50 % v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl

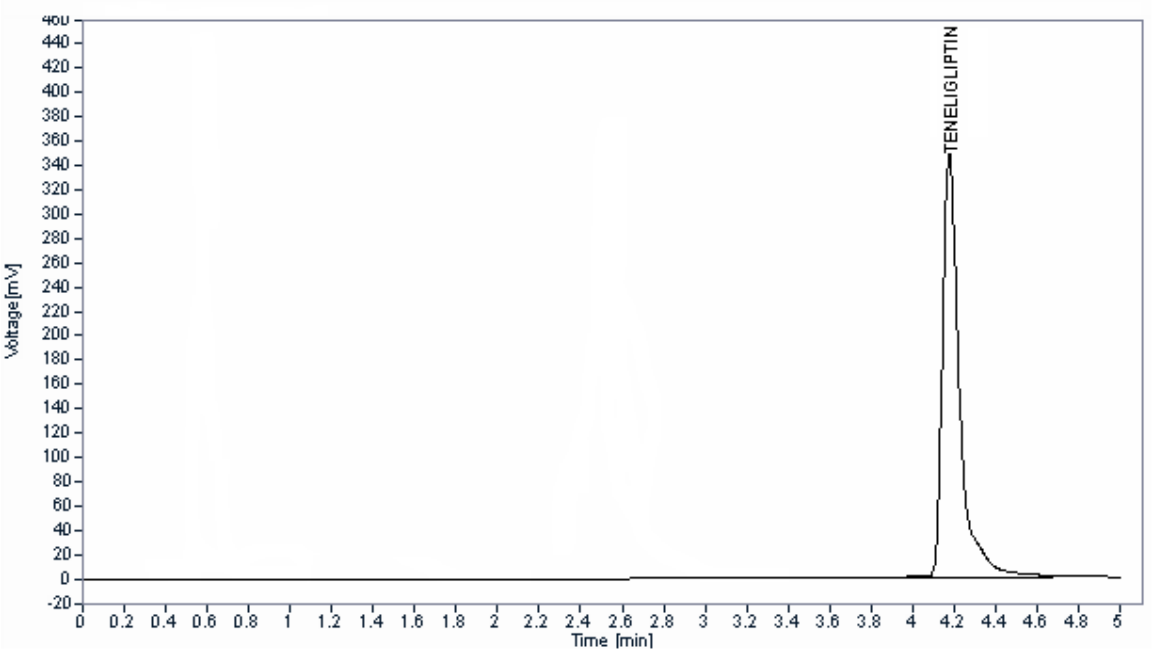


Observation: From the above chromatogram it was observed that the obtained Retention time, peak shape, tailing factor, theoretical plates is not upto the aim of the project.

TRIAL VII:

Chromatographic Conditions:

Mobile phase	:	Methanol: 1-Octane sulfonic acid Sodium salt pH 3.5(60:40% v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl

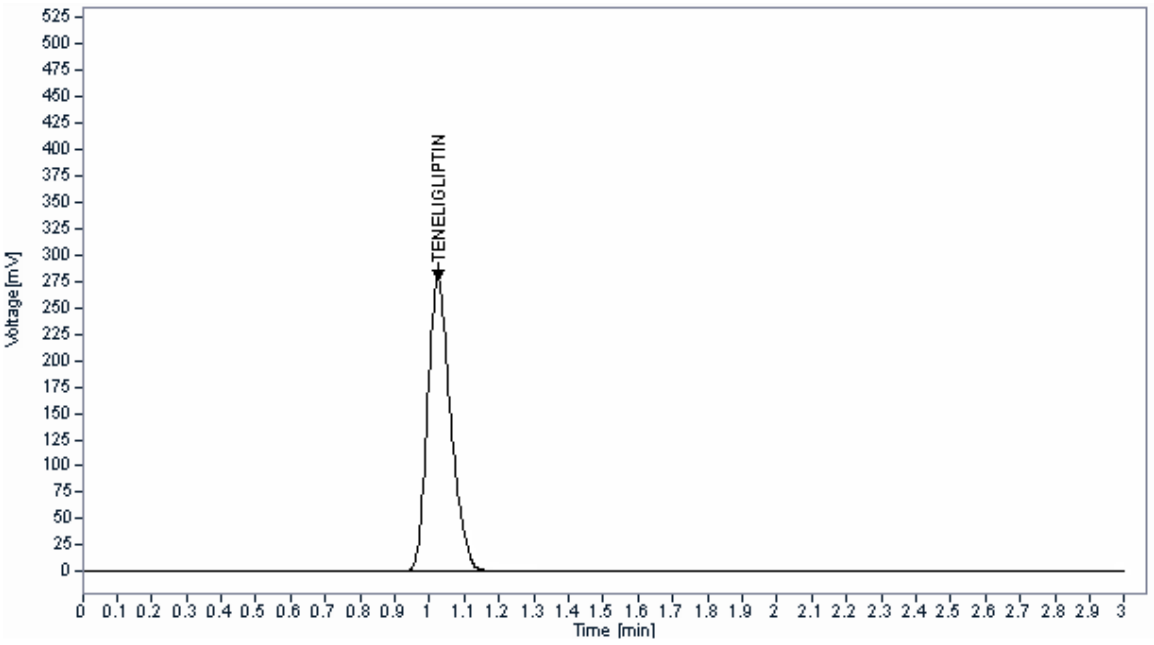


Observation: From the above chromatogram it was observed that the obtained Retention time, peak shape, tailing factor, theoretical plates is not upto the aim of the project.

TRIAL VIII:

Chromatographic Conditions:

Mobile phase	:	ACN: 1-Octane sulfonic acid Sodium salt pH 3.5 (50:50 % v/v)
Column	:	Phenomenex Luna C18 (50 mm x 3.0 mm, 3.0µm)
Flow rate	:	0.5 ml
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl



Observation: From the above chromatogram it was observed that the obtained Retention time, peak shape, tailing factor, theoretical plates,satisfies the aim of the project.

Hence this is optimized chromatographic conditions.

Optimized Chromatographic Conditions for Teneligliptin was		
Mobile phase	:	Buffer :ACN(50:50 v/v)
Buffer	:	1.0 g of 1-Octane sulfonic acid Sodium salt to 1000 ml of water. Adjust pH of solution to 3.5with dilute orthophosphoric acid
pH	:	3.5
Column	:	Phenomenex Luna C18 (50 mm x 3.0mm, 3.0µm)
Flow rate	:	0.5 ml/min
Wavelength	:	245 nm
Column Temperature	:	Ambient
Injection Volume	:	2µl
Run time	:	3 minutes

SYSTEM SUITABILITY:

1. Inject about 2 µL portion of standard solution into the chromatographic system five injections and measure the response of major peak.
2. The tailing factor for Teneligliptin peak should be NMT2.0

The RSD for the area of Teneligliptin peak obtained from the 5replicates injections of standard preparation should be NMT 2.0%.

Table No: 1

Injection	Retention time	Area	Theoretical plates	Tailing factor
BLANK	0.0	0	0	0.00
STANDARD-1	1.02	1361.354	2125	1.05
STANDARD-2	1.02	1365.524	2123	1.05
STANDARD-3	1.02	1364.443	2124	1.05
STANDARD-4	1.02	1363.557	2126	1.06
STANDARD-5	1.02	1364.756	2125	1.06
AVERAGE	-	1363.927	-	-
STDEV	-	1.602	-	-
%RSD	-	0.1	-	-

Fixed chromatographic conditions are as follows:

Instrument : UHPLC_AGILENT_1220 INFINITY LCwith UV detector and auto sampler.

Column : Phenomenex Luna C18, 50×3.0mm, 3µm particle size.

Flow rate : 0.5 mL/min

Wavelength : 245nm

Temperature : Ambient

Injection Volume : 2µL

Mobile phase composition: pH 3.5 Buffer: Acetonitrile (50:50V/v)

ASSAY METHOD VALIDATION

1. PRECISION

Precision covers

- System precision
- Method precision

System Precision

Six replicate injection of homogeneous standard solution indicate performance of UHPLC instrument underchromatographic conditions. Six sample preparations were prepared and injected at the nominal concentration level i.e., 100 ppm. The results obtained are as shown in Table-2

SYSTEM PRECISION

Table No: 2

Sample ID	Rt (min)	Area	Theoretical Plates (N)	Tailing Factor
SYSTEM PRECISION-1	1.02	1361.254	2125	1.05
SYSTEM PRECISION-2	1.02	1365.746	2153	1.03
SYSTEM PRECISION-3	1.03	1363.547	2145	1.06
SYSTEM PRECISION-4	1.02	1364.253	2136	1.05
SYSTEM PRECISION-5	1.02	1366.215	2162	1.05
SYSTEM PRECISION-6	1.02	1365.158	2135	1.06
AVERAGE	3.964	1364.362	2143	1.05
STDEV	-	1.807	-	-
%RSD	-	0.132	-	-
LIMITS	NMT 1%	NMT 2%	NLT 2000	NMT 2

Since the %RSD was found to be below 2%, the system precision parameter passed.

Method precision/Repeatability:

This is determined by preparing 9 samples of homogeneous sample mixture at 3 Concentrations and 3 replicates each and the results obtained are as shown in Table-3 and chromatogram follows.

METHOD PRECISION
Table No: 3

Test Preparation	Injection	Area	%Assay
Low Level	1	1090.24	99.35
	2	1090.05	99.05
	3	1090.34	99.82
Middle Level	1	1364.28	99.76
	2	1364.01	99.78
	3	1364.20	99.28
High Level	1	1635.00	99.43
	2	1634.56	99.64
	3	1634.19	99.17
Mean for Assay			99.48
Standard Deviation			0.285
%RSD			0.286

ACCURACY:

Dosage blends of Teneligliptin tablets was spiked with 10%,20%,and 30 % of known amount of Standard and to obtain110%, 120% and 130% with respect to labeled amount of drug in the formulation. It has been prepared in such a way that the average weight of tablet is kept constant and the weight of API was varied. Test solution was injected and the assay was performed as per the test method. From this "%Recovery" and mg recovered were calculated. The results are as shown in the following Table No: 4 and follows chromatograms.

$$\% \text{ Recovery} = \frac{\text{Sample peak area}}{\text{Standard peak area}} \times \frac{\text{Dilution factor of standard}}{\text{Dilution factor of sample}} \times \text{Potency} \times 100$$

ACCURACY

Table No: 4

Spiked Level	API Added (mg)	API Recovered (mg)	%Recovery
110 %	2.0001	1.9917	99.58
110 %		1.9919	99.59
110 %		1.9943	99.71
120%	4.0002	3.9804	99.50
120%		3.9925	99.81
120 %		3.9943	99.85
130 %	6.0004	6.0040	100.06
130%		5.9902	99.83
130 %		5.9819	99.69

Acceptance criteria here are that the % Recovery calculated should be between 98% to 102%. Here percentage Recovery is calculated using the above formula.

LINEARITY

Linearity has been demonstrated in the range of 50-150% of target concentration of the assay. Five series of standard solutions in the above concentration range were injected in duplicate. Standard solution in concentrations of 50, 70, 100, 130, 150 ppm were prepared and injected and the peaks areas and other statistical data are given in Table No: 5. A calibration curve was determined for the drug independently by plotting

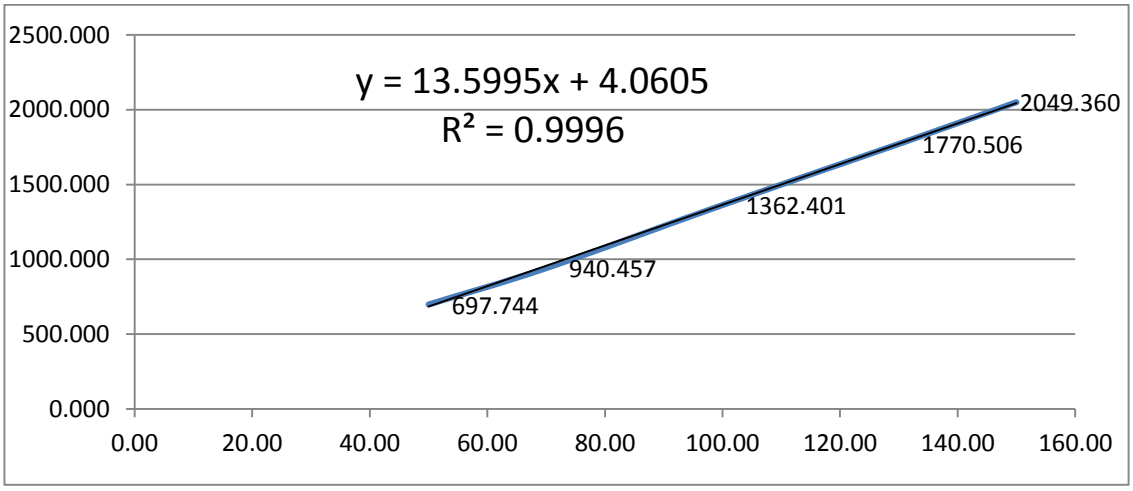
the peak areas obtained against concentrations (in percentage). There exists a linear relationship in the two graphs for the two concentration ranges which are prepared. From the data obtained correlation coefficient, Y- intercept and slope were calculated to provide mathematical estimates of the degree of linearity. Chromatograms are as shown below,

From the chromatograms, average area of Teneligliptin is calculated and is tabulated as follows. From this response values a linear curve is obtained as shown in below.

Table No: 5

LINEARITY CURVE FOR TENELIGLIPTIN

API Percentage					
	50%	**70%**	**100%**	**130%**	**150%**
Trial-1	697.839	940.541	1362.465	1770.863	2049.276
Trial-2	697.648	940.374	1362.337	1770.148	2049.443
Average	697.744	940.458	1362.401	1770.506	2049.360



SPECIFICITY:

Placebo Interference:

Assay was performed on the weight of the placebo equivalent to the amount present in the test preparation. Chromatogram of the placebo did not show any peak at the Retention time of the analyte peak. So interference of the placebo is considered significant. Chromatograms are as follows,

Table No: 6

Sample No.	% Interference
1	Nil

RUGGEDNESS/INTERMEDIATE PRECISION

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, analysts, instruments, lots of reagents, elapsed assay times, assay temperatures or days. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from analyst to analyst, system to system.

a) Different Days

In this, criteria analyst carried the analysis on different days. The result is calculated as % of labeled amount of drug substance, RSD of assay results. It is said to be rugged if the assay result is within 98%-102% and the % RSD should be NMT 2%. The data results are being shown in Table-7

TABLE-7

Sample no	Assay of Teneligliptin as % of labeled amount	
	Day-01	Day-02
1	99.68	99.69
2	99.22	99.69
3	99.43	99.13
4	99.13	99.83

5	99.89	99.06
6	99.98	99.62
Average	99.55	99.50
%RSD	0.35	0.33

Acceptance criteria

The all individual assays of Teneligliptin should be within 98% to 102%. Relative standard deviation of % assay results should not be more than 2.0% on two days.

ROBUSTNESS:

Robustness examines the effect of variation in operational parameters on the analysis results. For the determination of a method's robustness, chromatographic parameters like mobile phase pH, mobile phase composition, flow rate, column temperature and variation in columns are varied within a realistic range and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range.

Measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides indication of its reliability during its normal usage.

Varying method parameters within a realistic range and the quantitative influence of the variables was determined, and, if the influence of the parameter was within a previously specified tolerance, then, the parameter was said to be within the method's robustness range.

Typical variations includes

- Flow rate
- Altered mobile phase Ratio

Table No: 8
Altered Flow rate

Sample no	Assay of Teneligliptin as % of labeled amount			
	Low Flow rate 0.4 ml		High Flow rate 0.6 ml	
	RT	%	RT	%
1	1.32	99.69	0.86	99.80
2	1.32	99.75	0.86	100.09
3	1.32	99.36	0.86	99.21
4	1.32	99.64	0.86	99.72
5	1.32	100.05	0.86	99.67
6	1.32	99.16	0.86	99.44
Average		99.61		99.65
%RSD		0.31		0.31

Table No: 9
Altered Organic Phase ratio

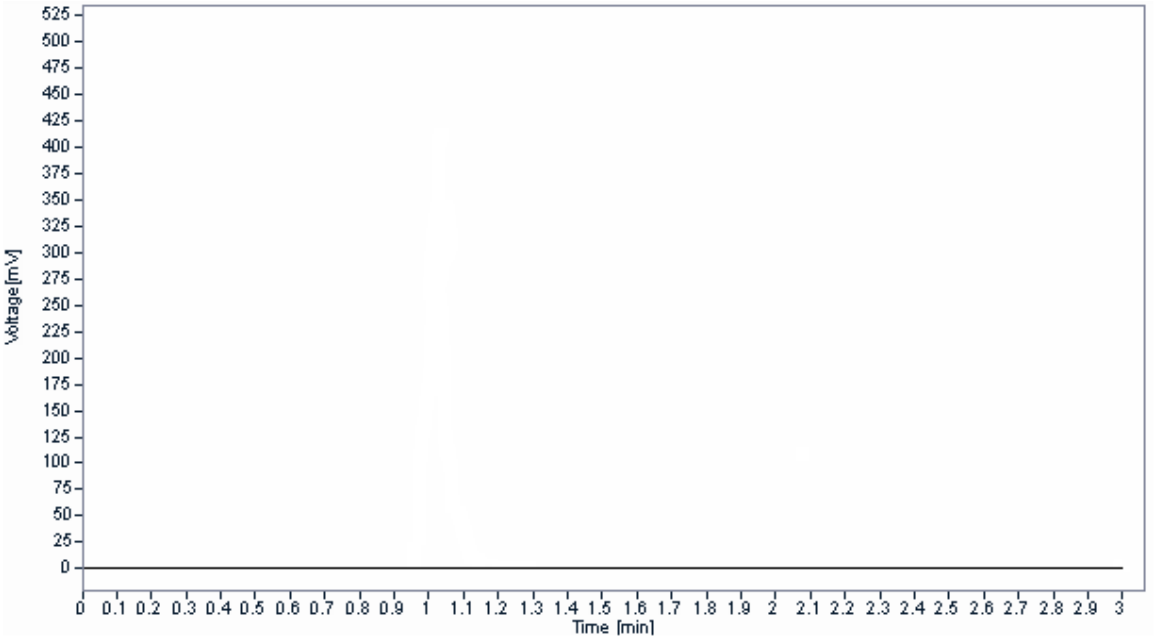
Sample no	Assay of Teneligliptin as % of labeled amount			
	Low Organic Ratio (Buffer:ACN – 60:40)		High Organic Ratio (Buffer:ACN – 40:60)	
	RT	%	RT	%
1	1.45	99.09	0.91	99.66
2	1.45	99.23	0.91	99.87
3	1.45	99.43	0.91	98.73
4	1.45	99.49	0.91	100.08
5	1.45	99.37	0.91	99.20
6	1.45	99.19	0.91	99.11
Average		99.30		99.44
%RSD		0.16		0.51

SYSTEM SUITABILITY

Flow rate: 0.5 mL/min Injection volume: 2µL

Wave length: 245nm Temperature: Ambient

SYSTEM SUITABILITY: BLANK



Graph No- 01

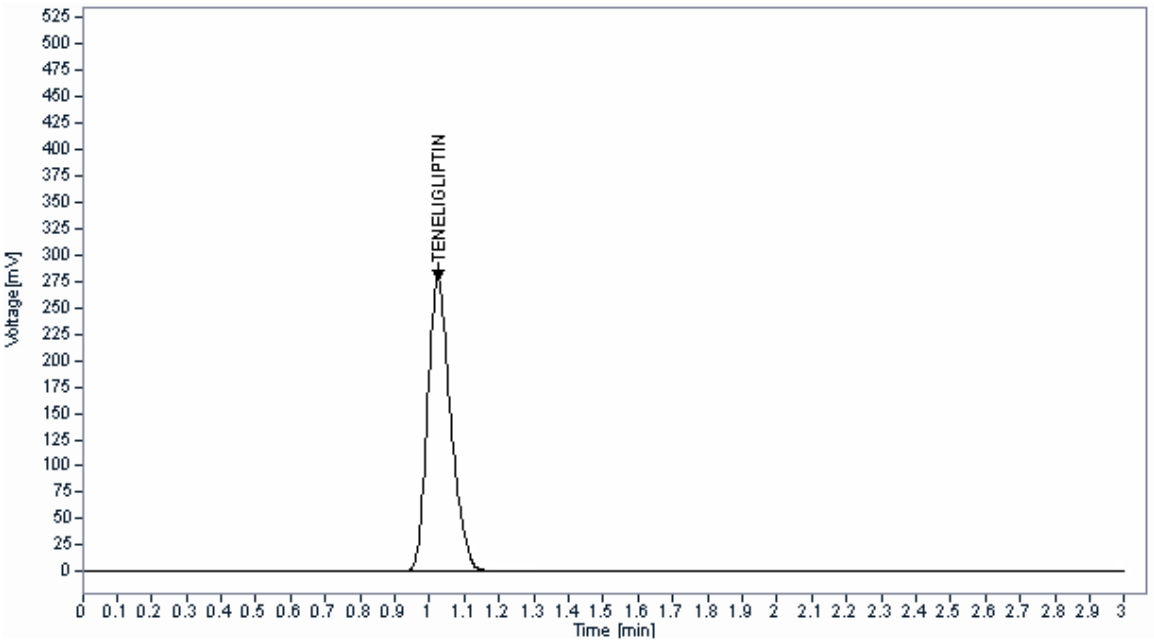
Injection	Retention time	Area	Theoretical plates	Tailing factor
BLANK	0.0	0	0	0.00

SYSTEM SUITABILITY

Flow rate: 0.5 mL/min Injection volume: 2µL

Wave length: 245nm Temperature: Ambient

SYSTEM SUITABILITY: STANDARD-1



Graph No- 02

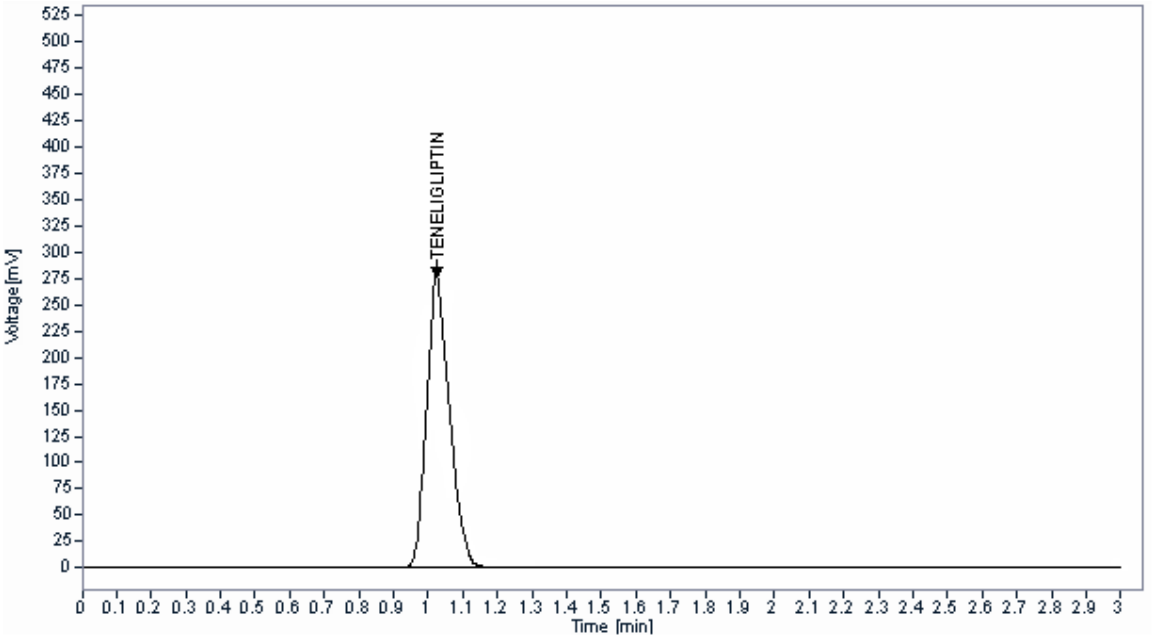
Injection	Retention time	Area	Theoretical plates	Tailing factor
STANDARD-1	1.02	1361.354	2125	1.05

SYSTEM SUITABILITY

Flow rate: 0.5 mL/min Injection volume: 2µL

Wave length: 245nm Temperature: Ambient

SYSTEM SUITABILITY: STANDARD-2



Graph No- 03

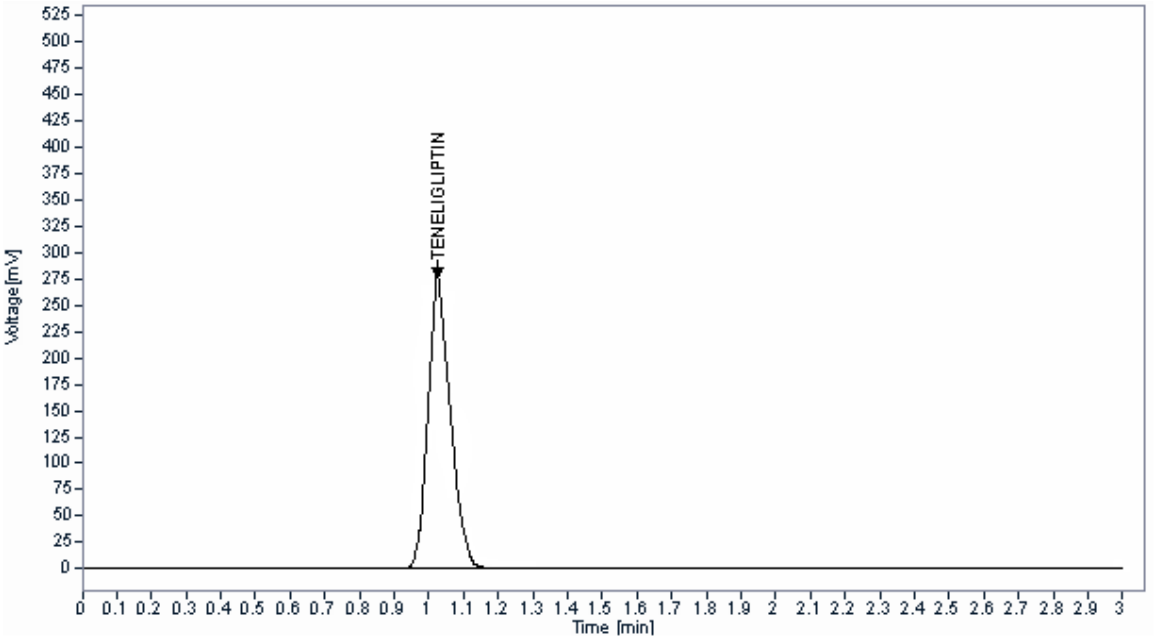
Injection	Retention time	Area	Theoretical plates	Tailing factor
STANDARD-2	1.02	1365.524	2123	1.05

SYSTEM SUITABILITY

Flow rate: 0.5 mL/min Injection volume: 2µL

Wave length: 245nm Temperature: Ambient

SYSTEM SUITABILITY: STANDARD-3



Graph No- 04

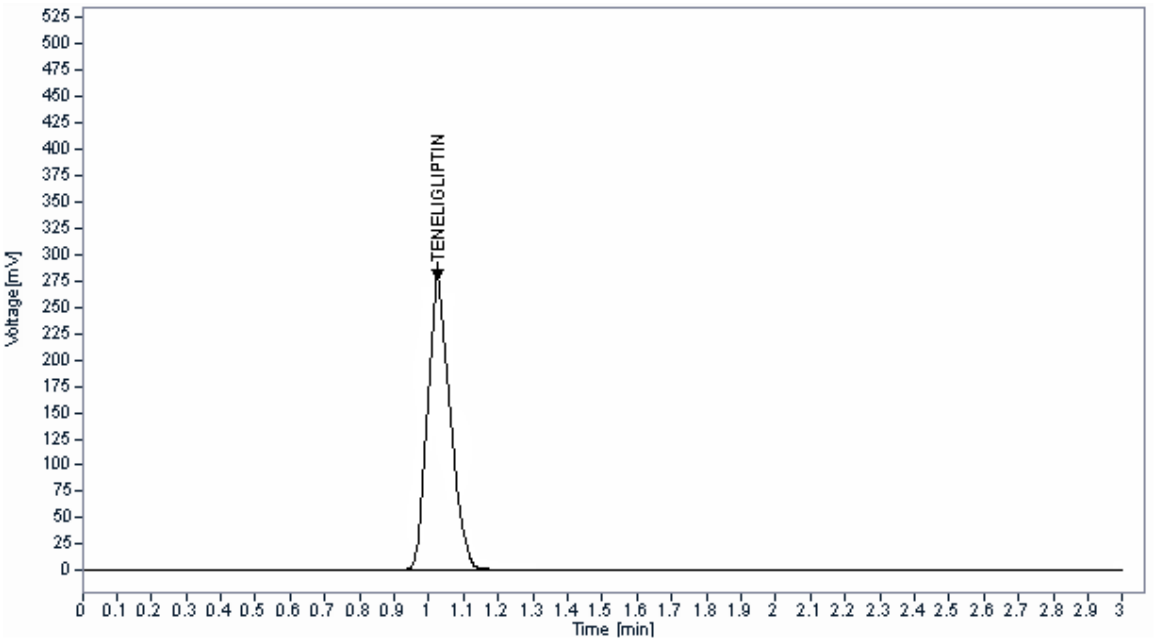
Injection	Retention time	Area	Theoretical plates	Tailing factor
STANDARD-3	1.02	1364.443	2124	1.05

SYSTEM SUITABILITY

Flow rate: 0.5 mL/min Injection volume: 2µL

Wave length: 245nm Temperature: Ambient

SYSTEM SUITABILITY: STANDARD-4



Graph No- 05

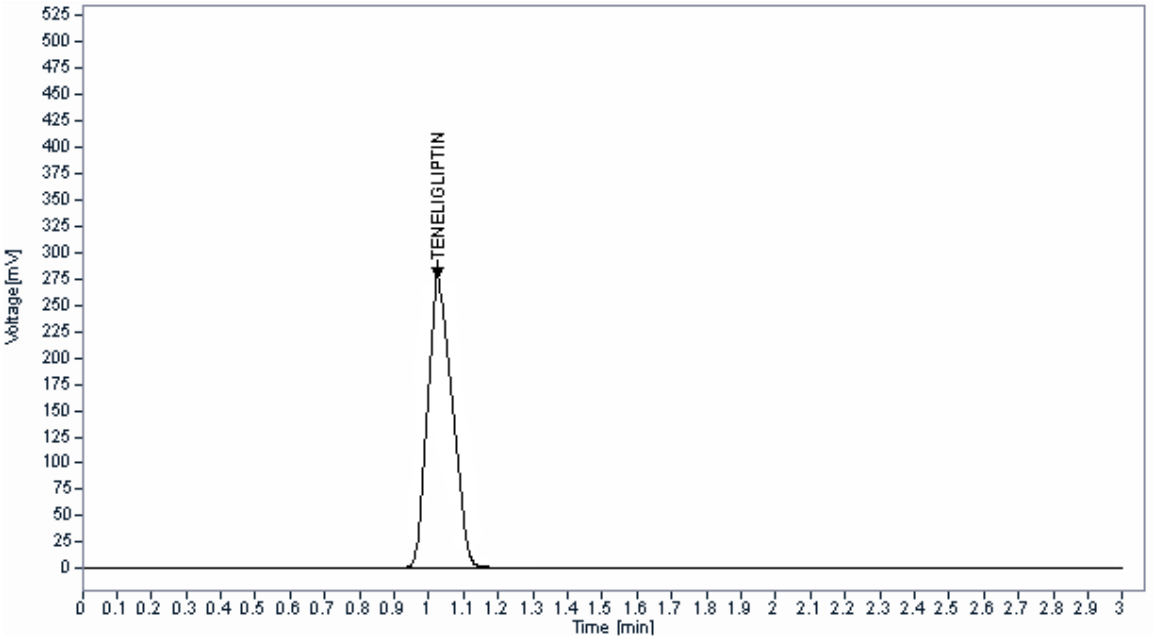
Injection	Retention time	Area	Theoretical plates	Tailing factor
STANDARD-4	1.02	1363.557	2126	1.06

SYSTEM SUITABILITY

Flow rate: 0.5 mL/min Injection volume: 2µL

Wave length: 245nm Temperature: Ambient

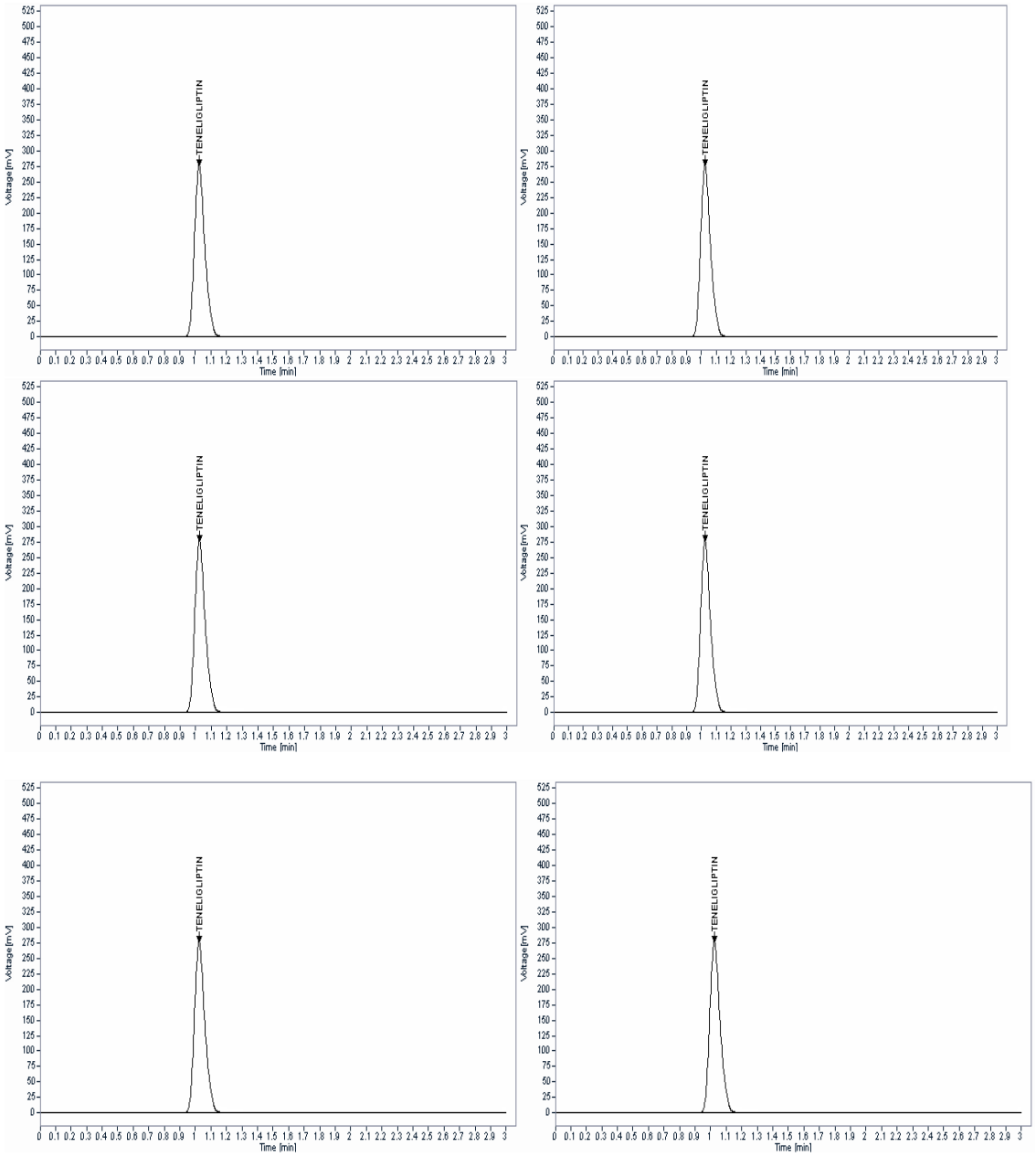
SYSTEM SUITABILITY: STANDARD-5



Graph No- 06

Injection	Retention time	Area	Theoretical plates	Tailing factor
STANDARD-5	1.02	1364.756	2125	1.06

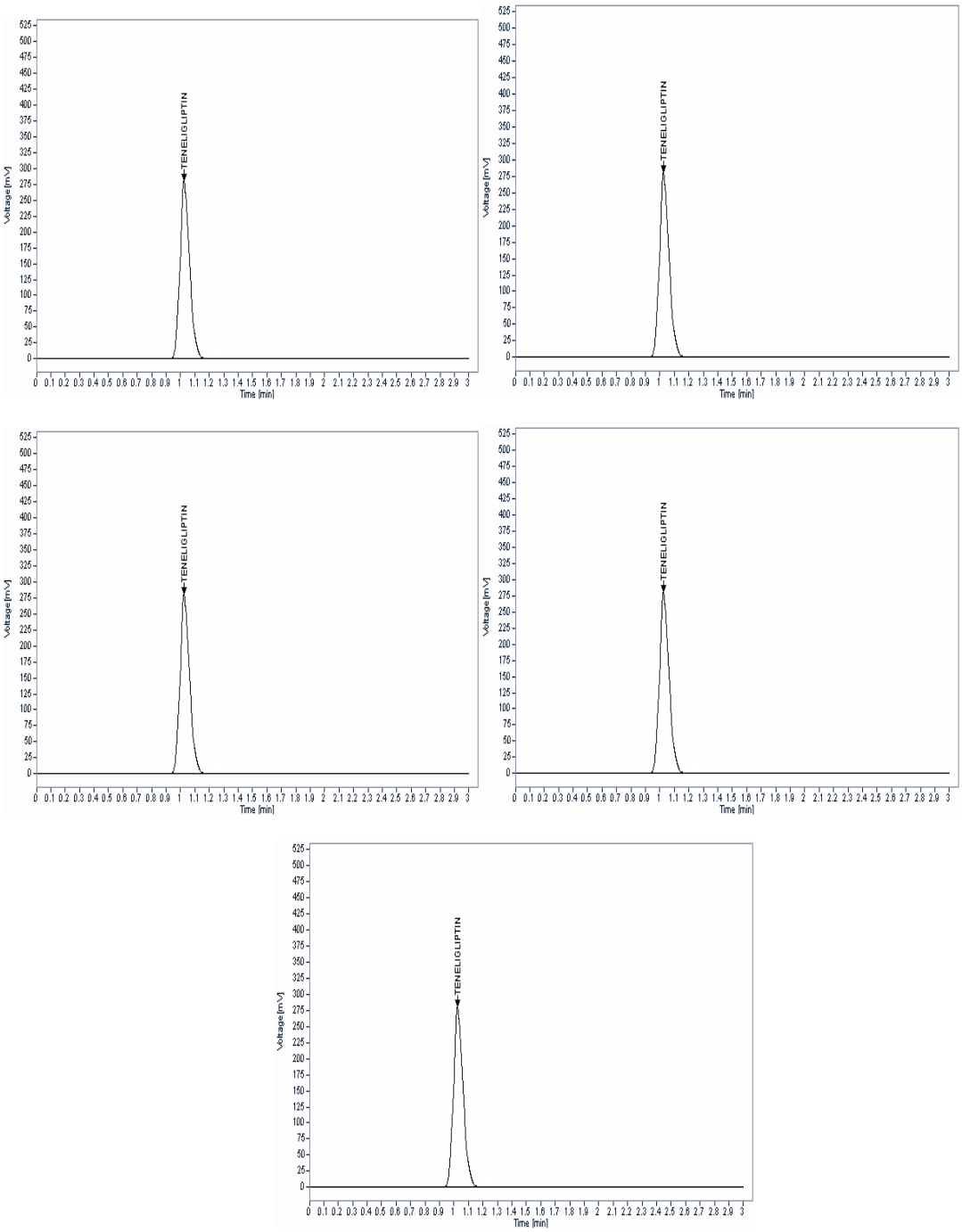
SYSTEM PRECISION



Graph No- 07

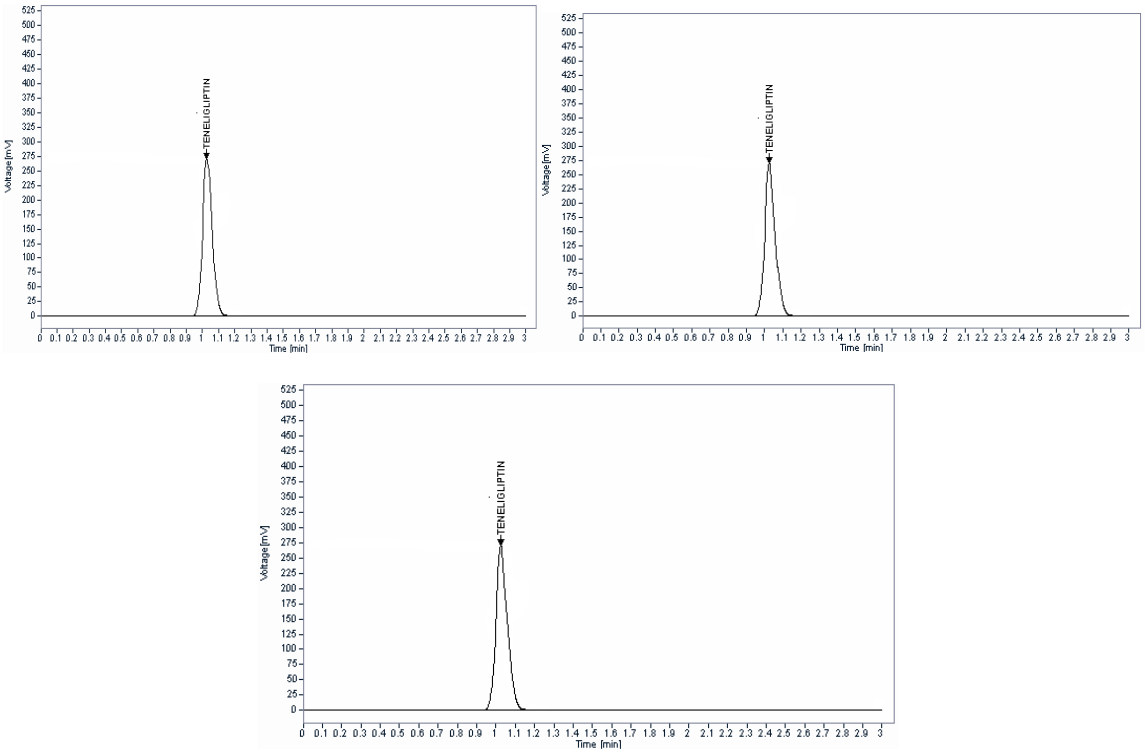
METHOD PRECISION

STANDARD CHROMATOGRAMS



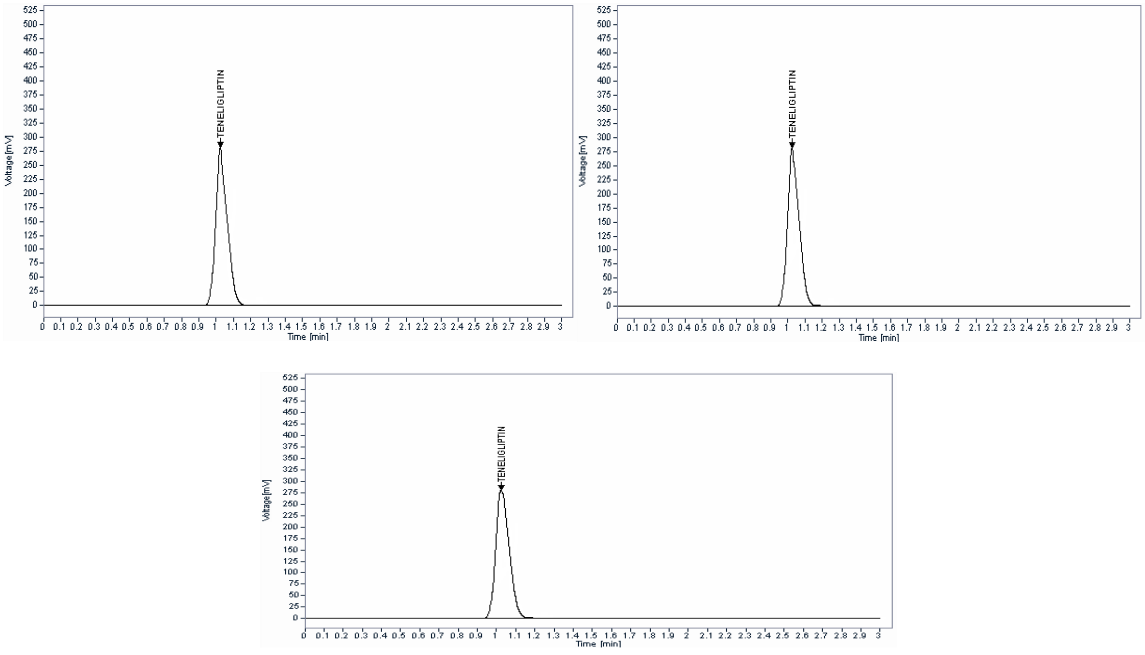
Graph No- 08

LOW LEVEL (80 %)



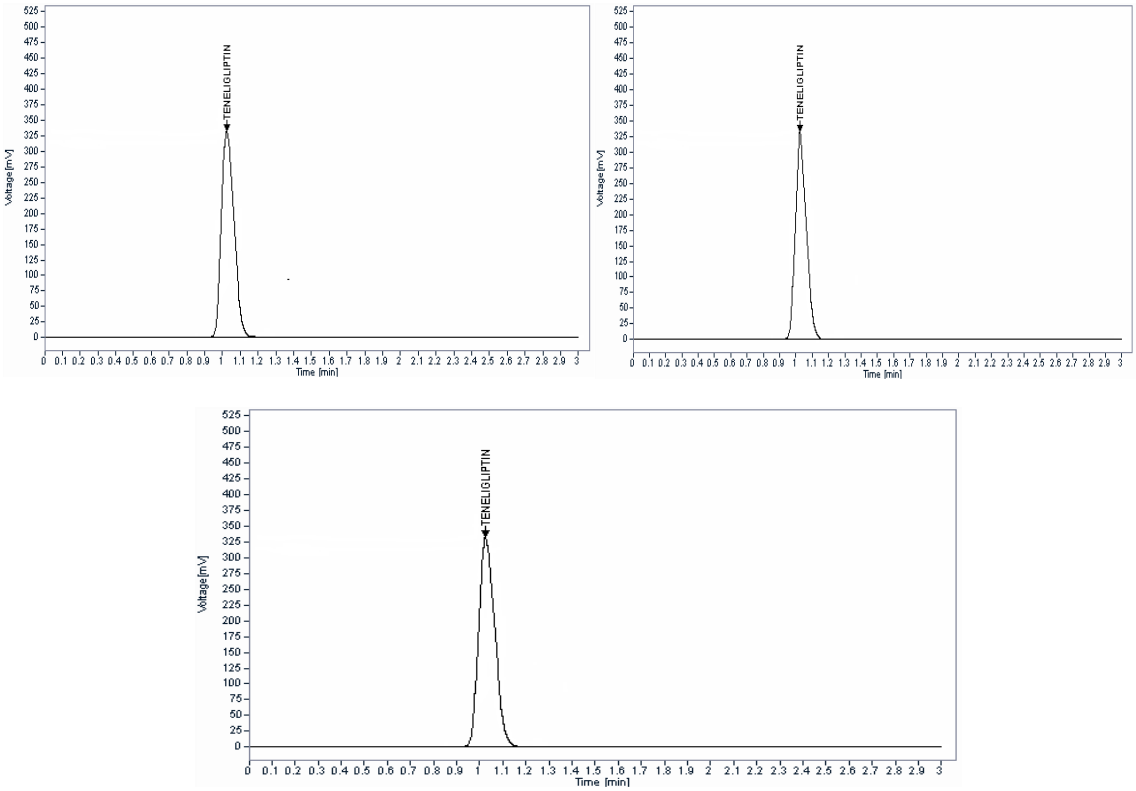
Graph No- 09

MIDDLE LEVEL (100 %)



Graph No- 10

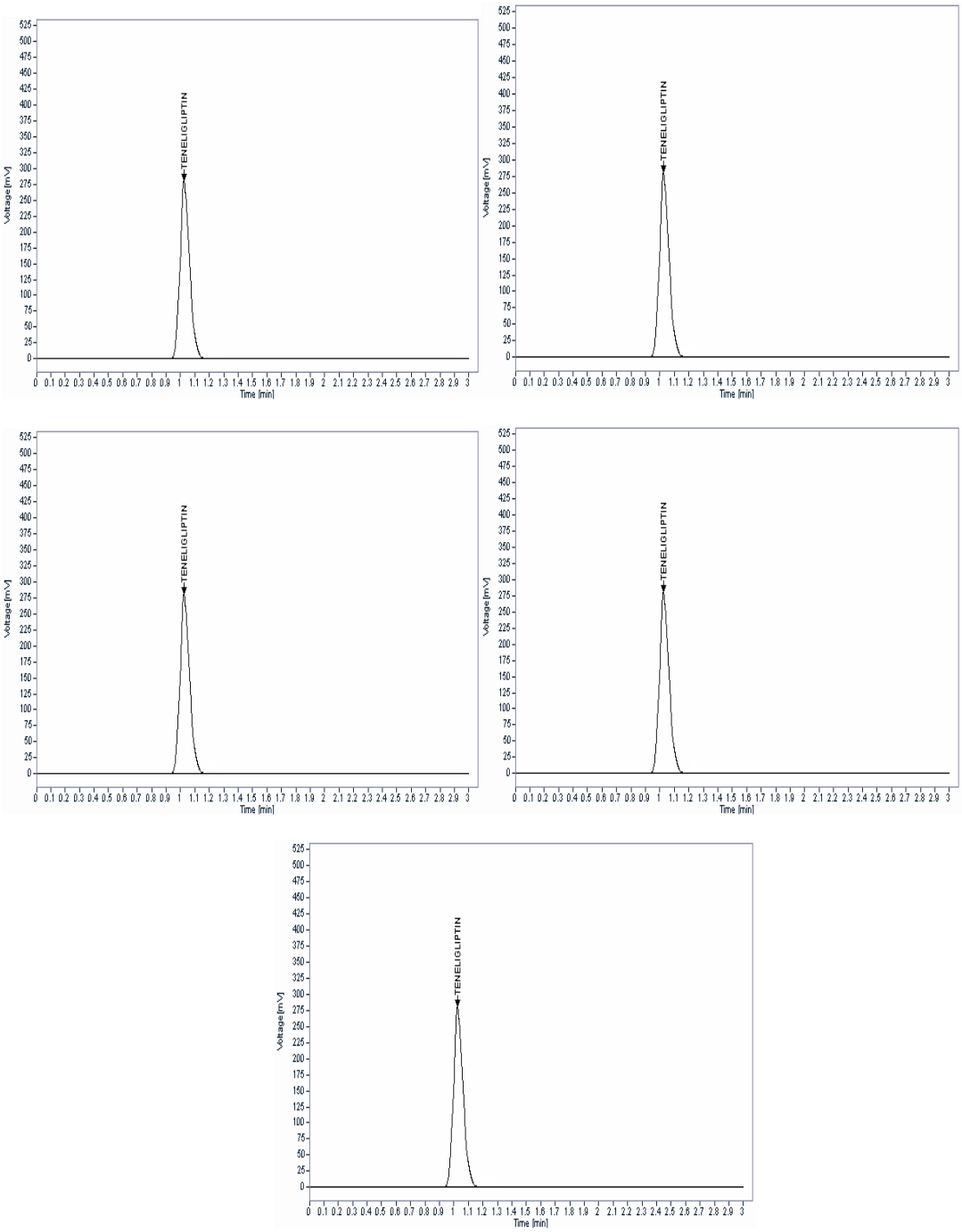
HIGH LEVEL (120 %)



Graph No- 11

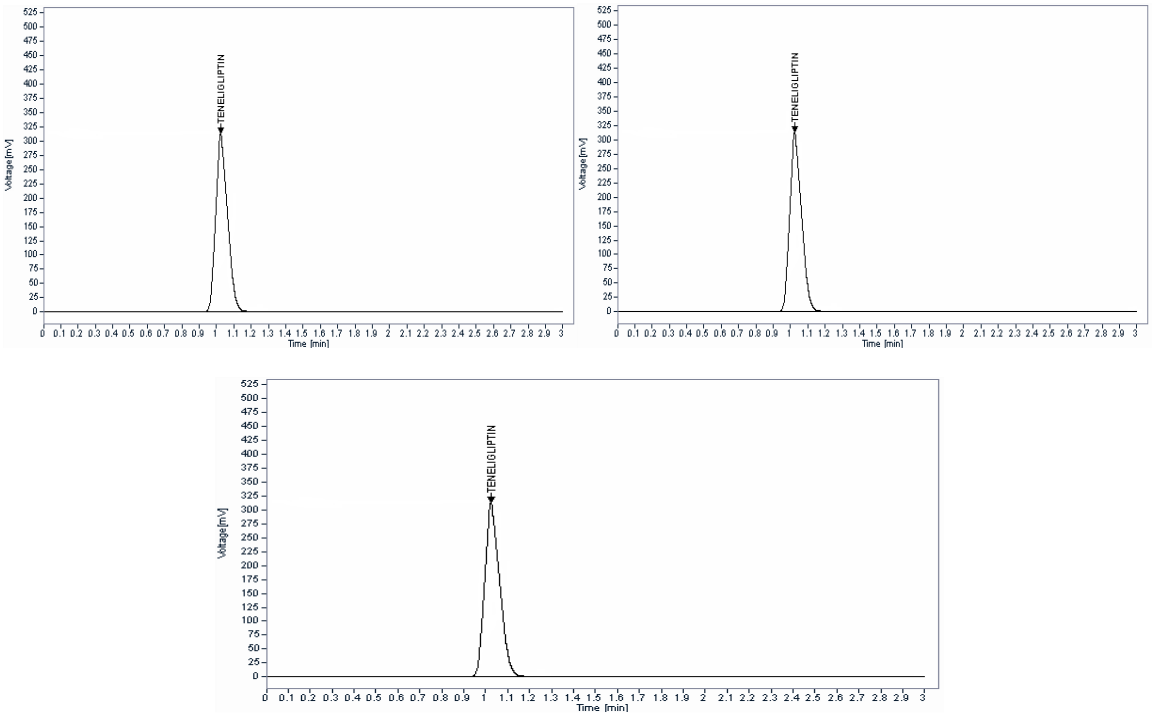
ACCURACY (% Recovery)

STANDARD CHROMATOGRAMS



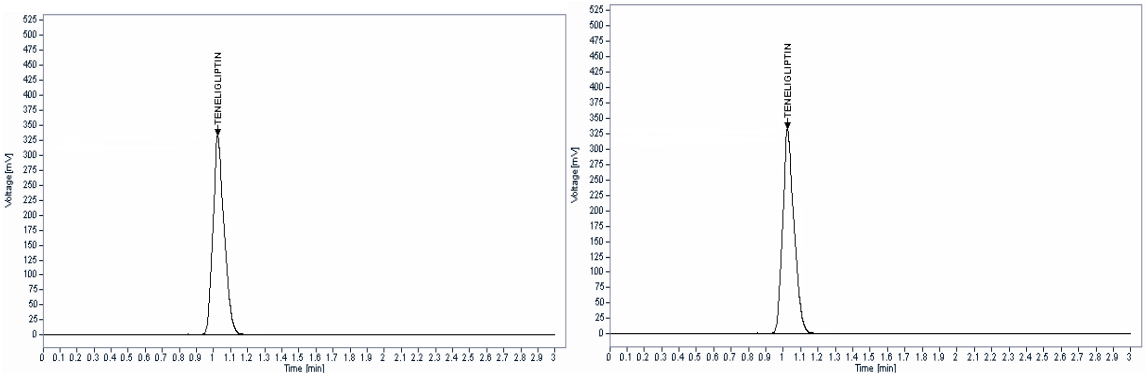
Graph No- 12

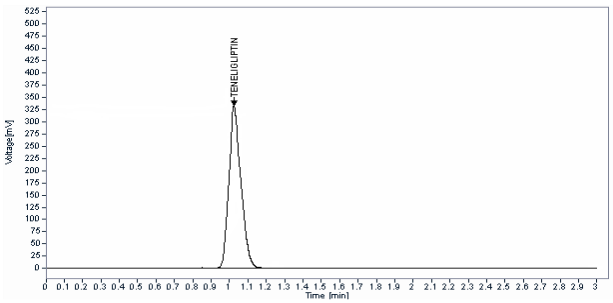
Spike Level 10 %



Graph No- 13

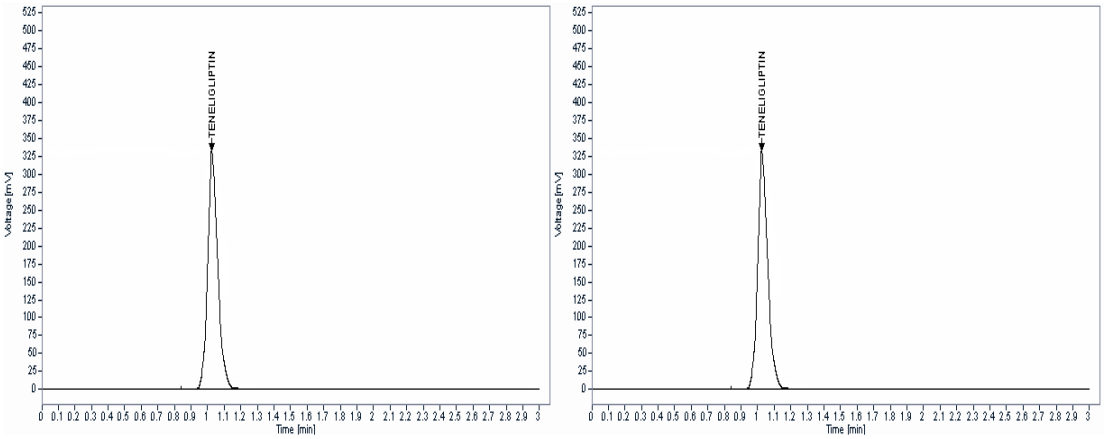
Spike Level 20 %

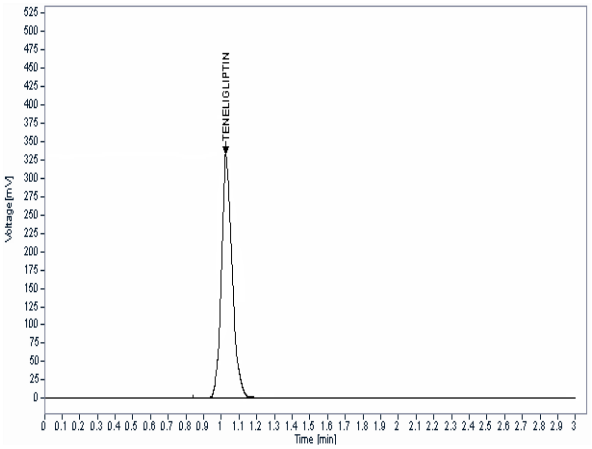




Graph No- 14

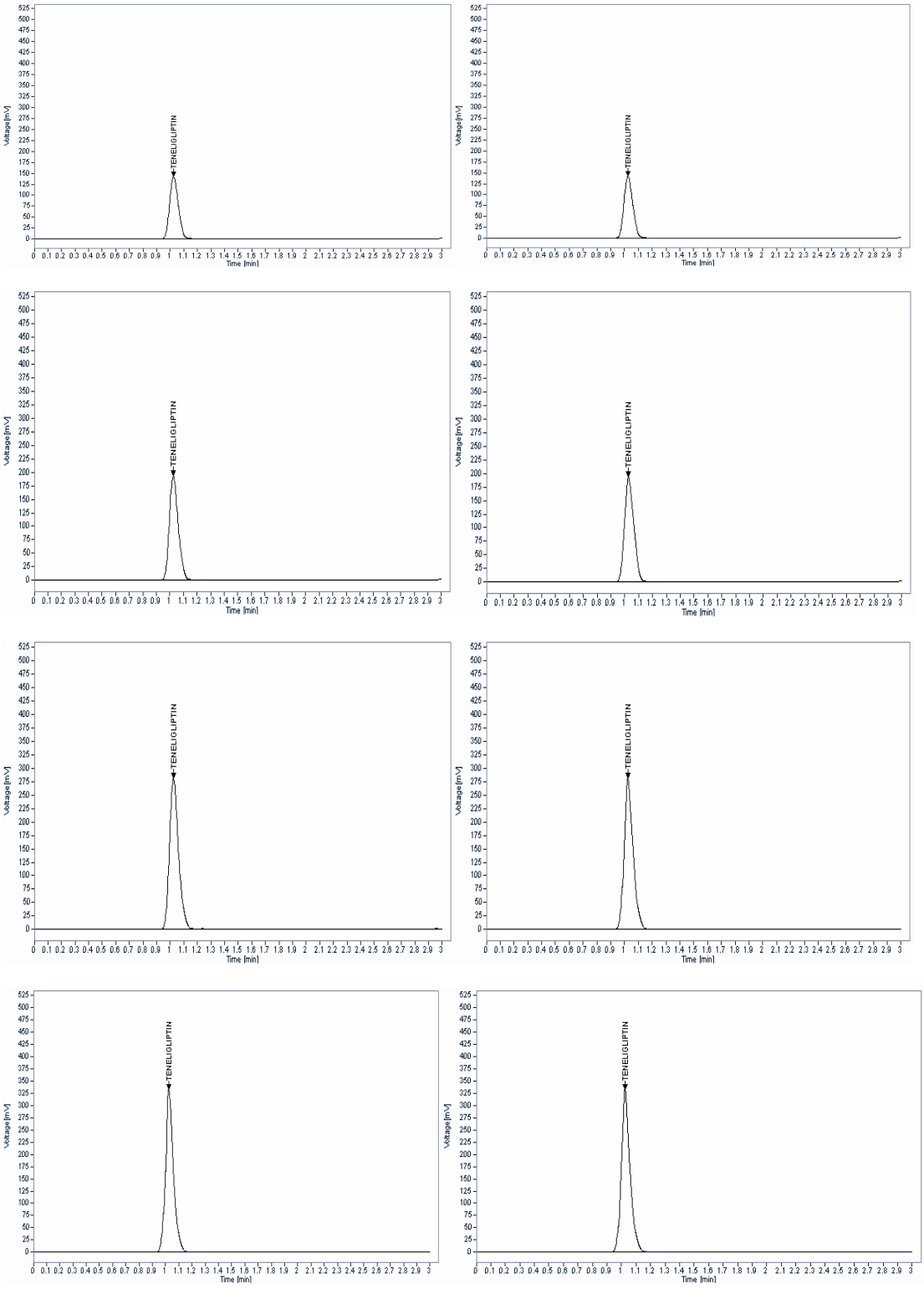
Spike Level 30 %



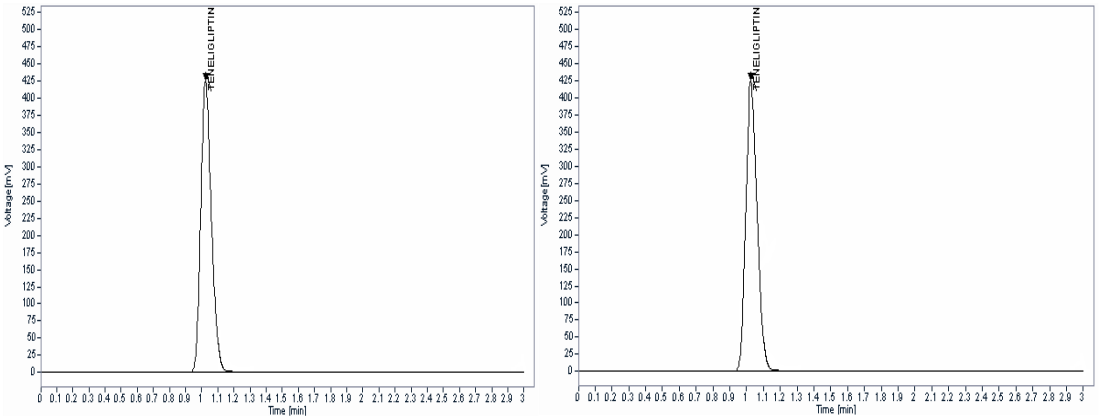


Graph No- 15

LINEARITY CHROMATOGRAMS

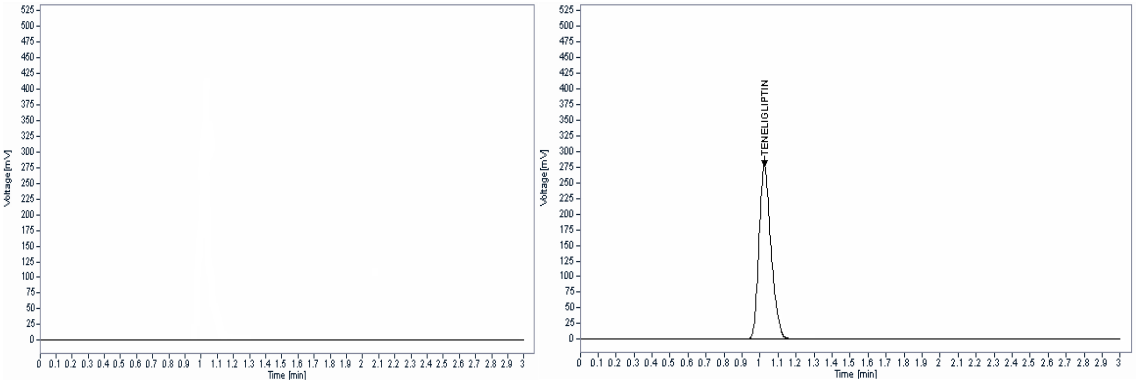


LINEARITY CHROMATOGRAMS



Graph No- 16

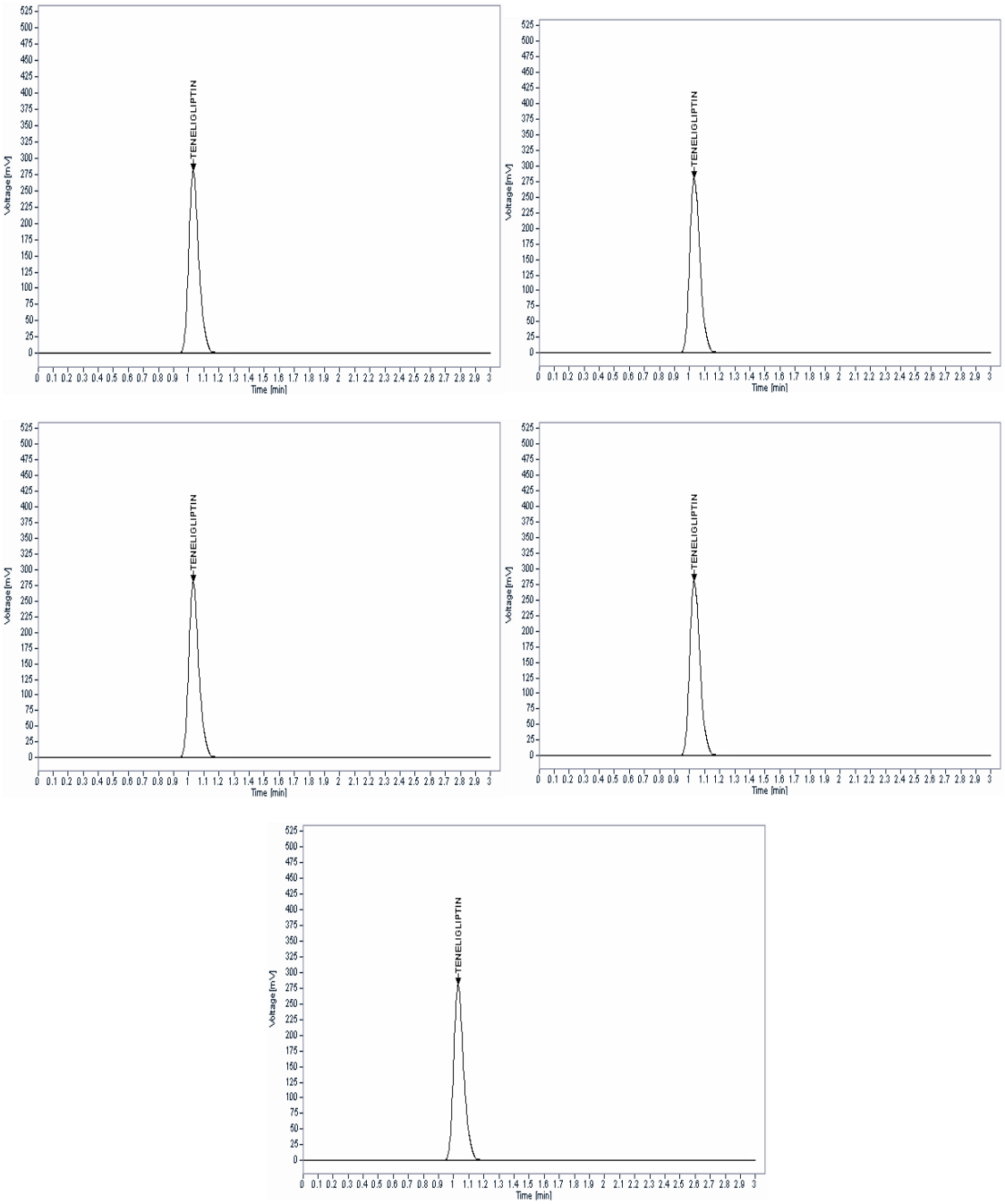
SPECIFICITY



Graph No- 17

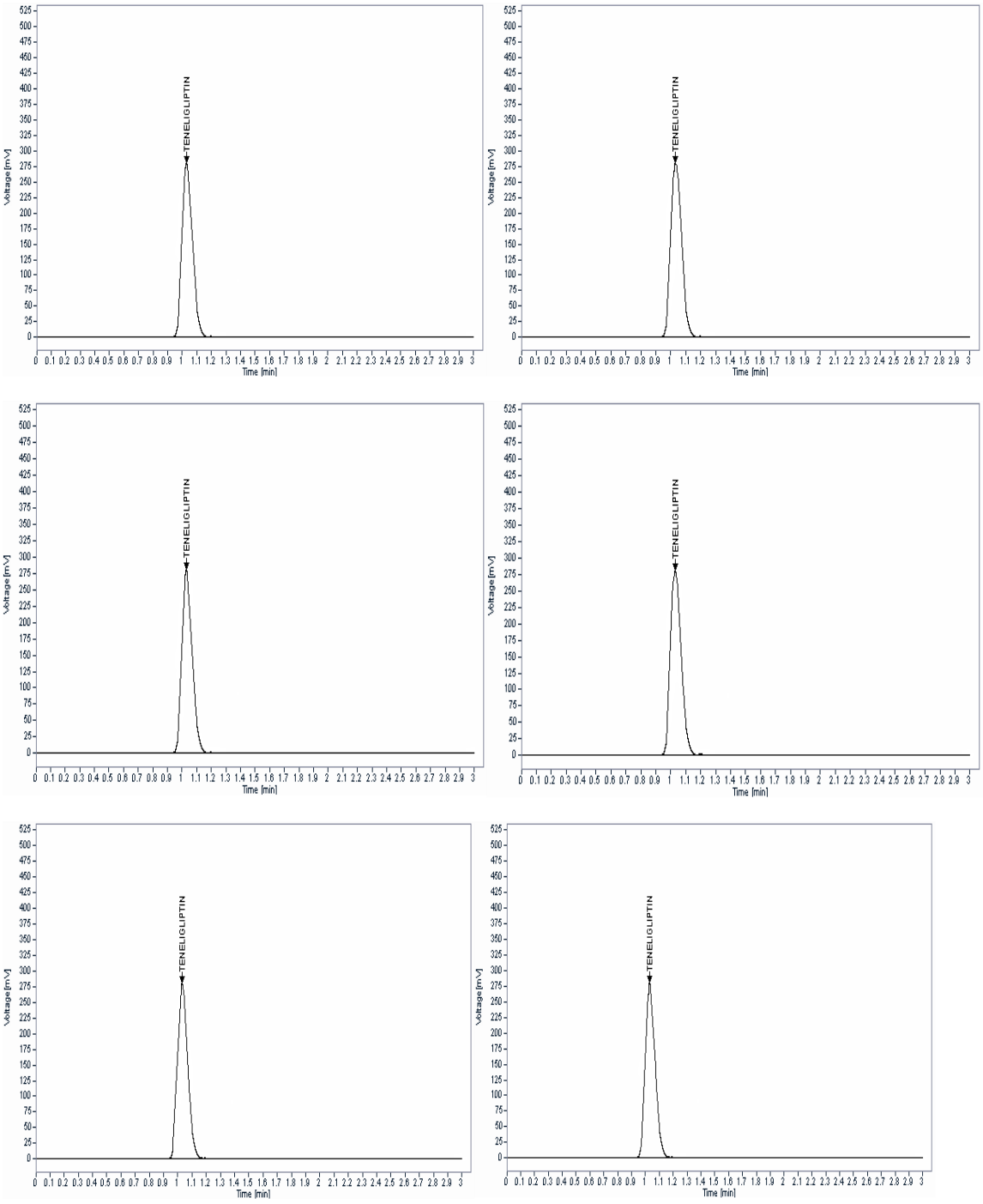
RUGGEDNESS

DAY-01 STANDARD CHROMATOGRAMS



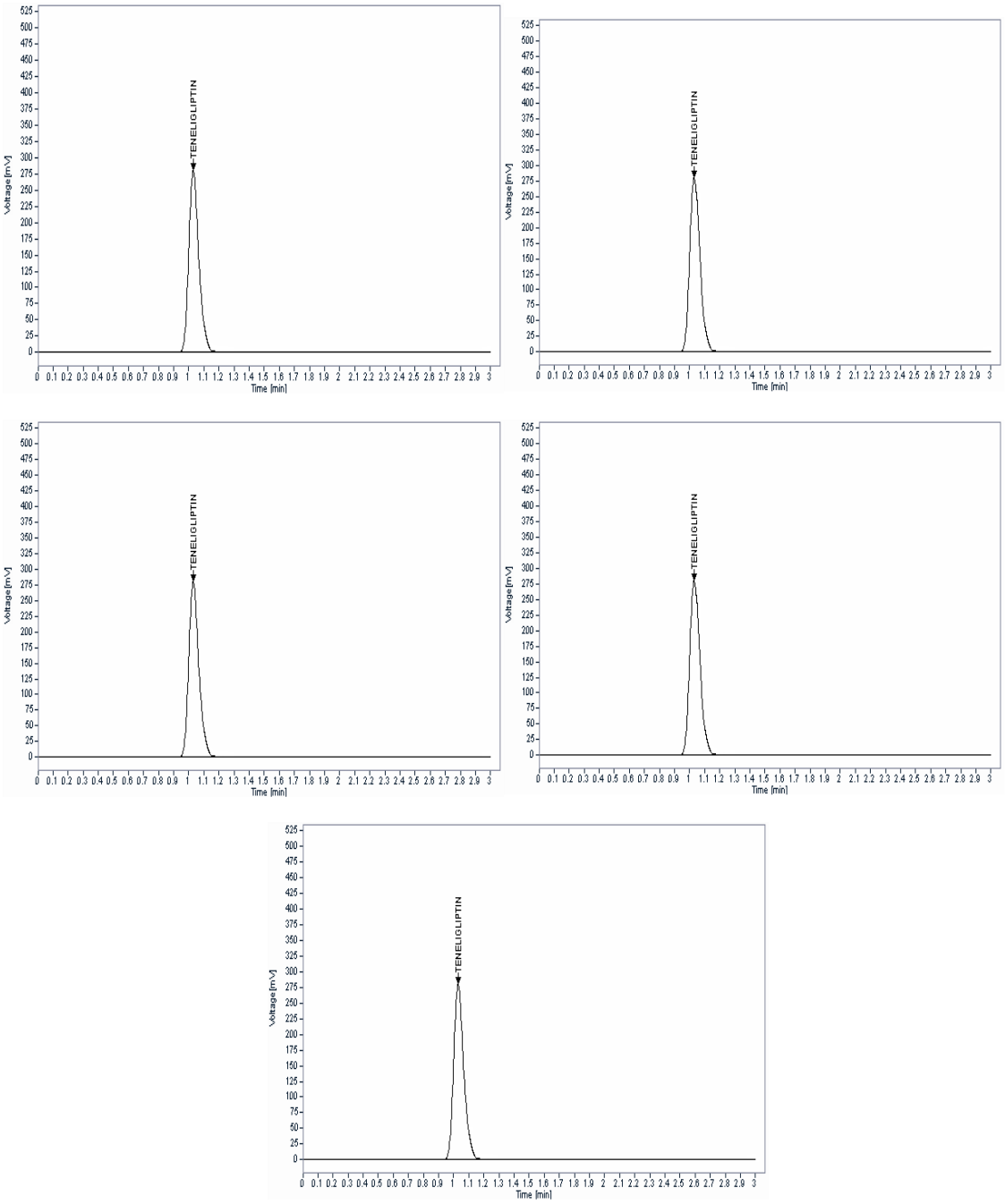
Graph No- 18

DAY-01 SAMPLE CHROMATOGRAMS



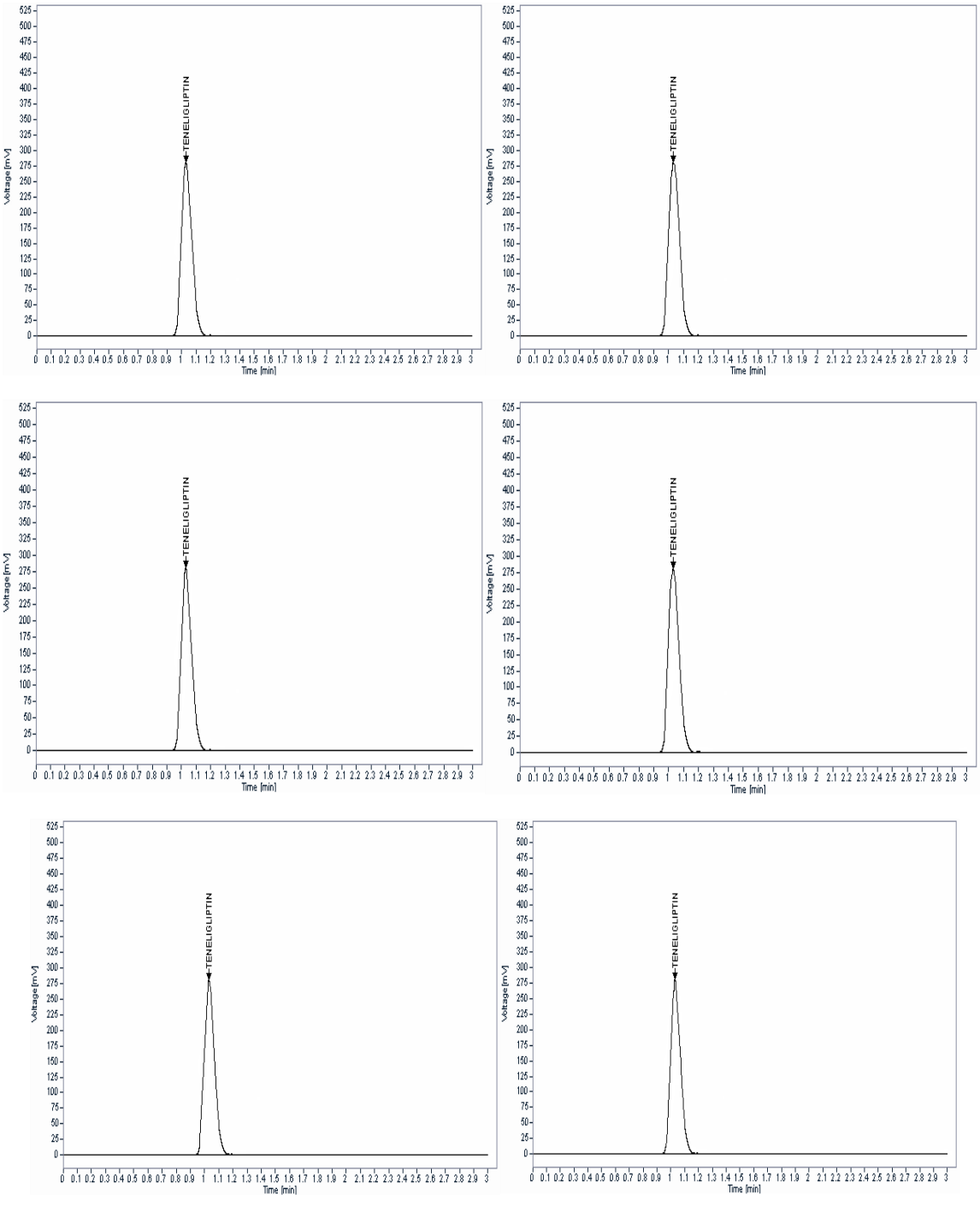
Graph No- 19

DAY-02 STANDARD CHROMATOGRAMS



Graph No- 20

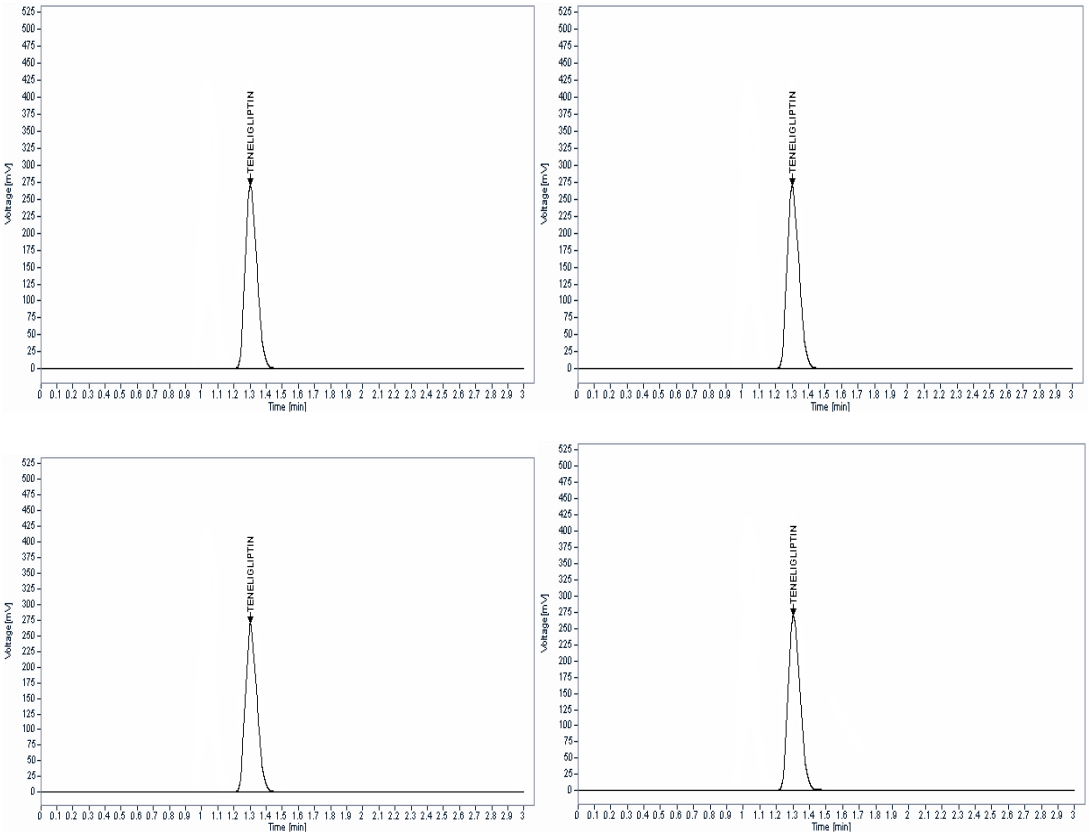
DAY-02 SAMPLE CHROMATOGRAMS

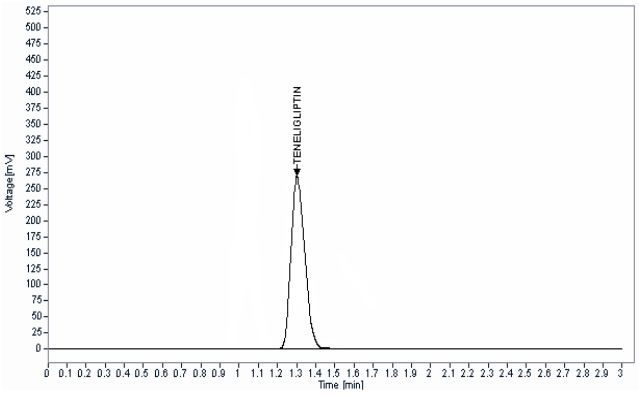


Graph No- 21

ROBUSTNESS

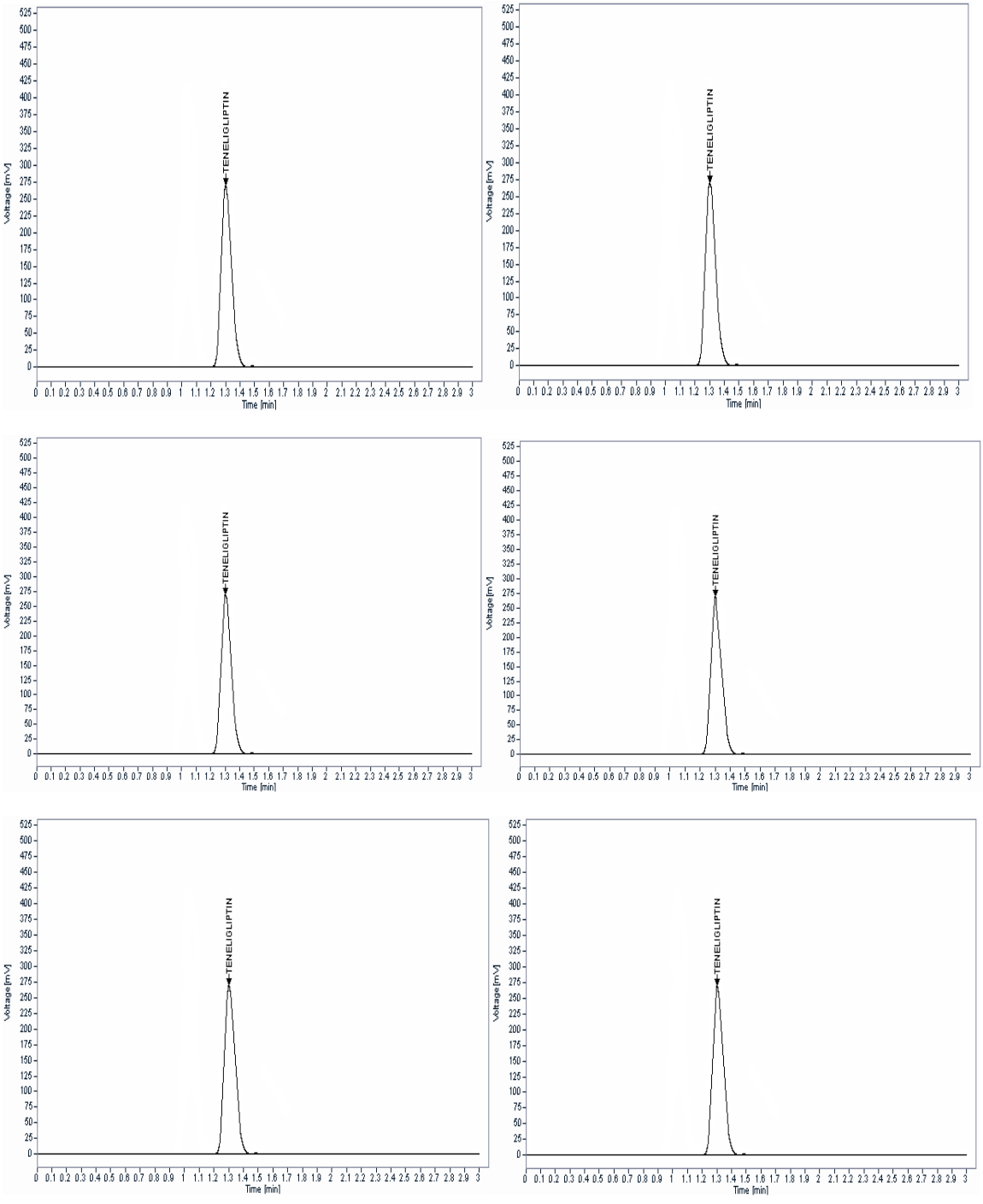
LOW FLOW RATE (0.4 ml) STANDARD CHROMATOGRAMS





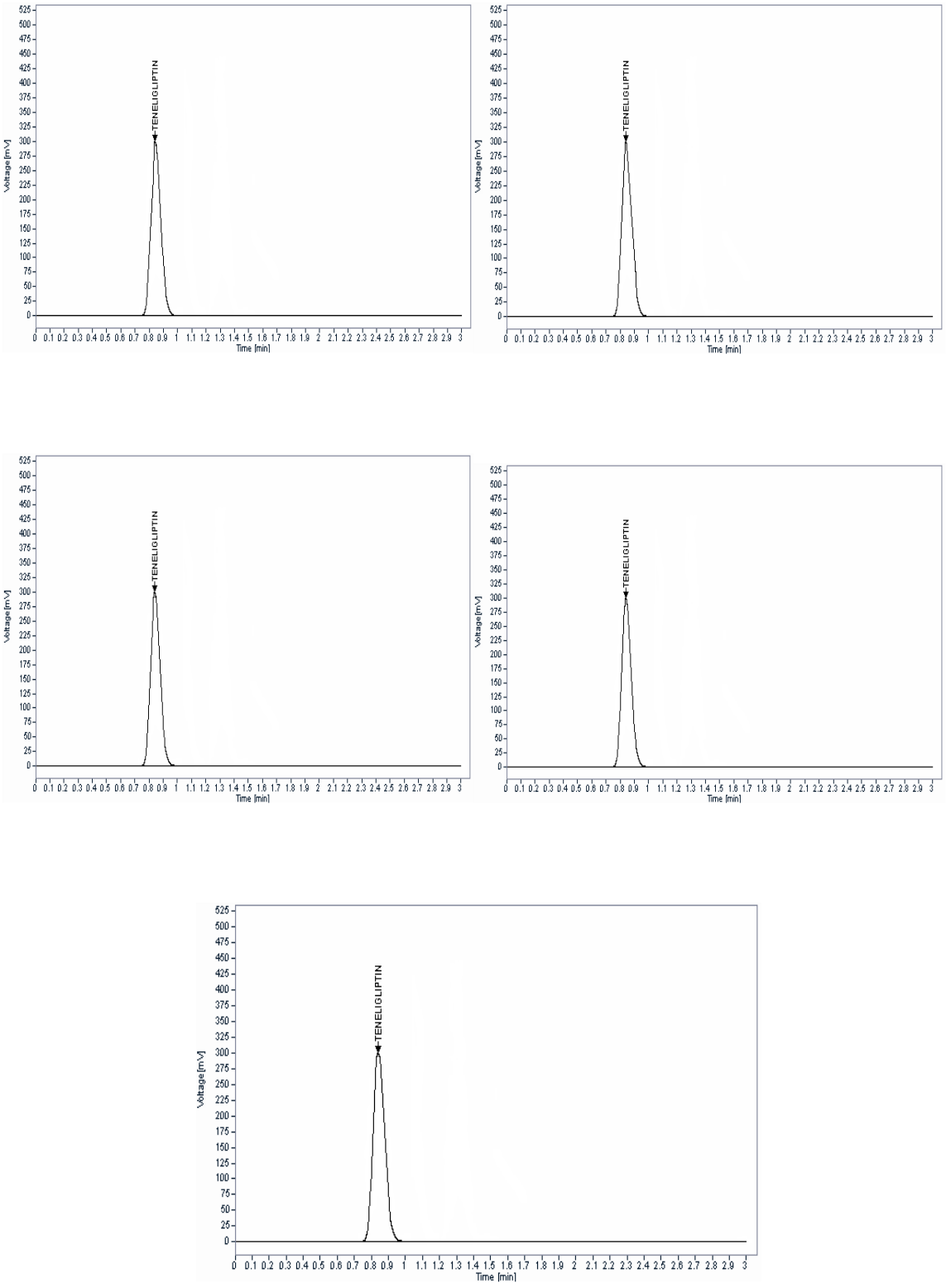
Graph No- 22

LOW FLOW RATE (0.4 ml) SAMPLE CHROMATOGRAMS



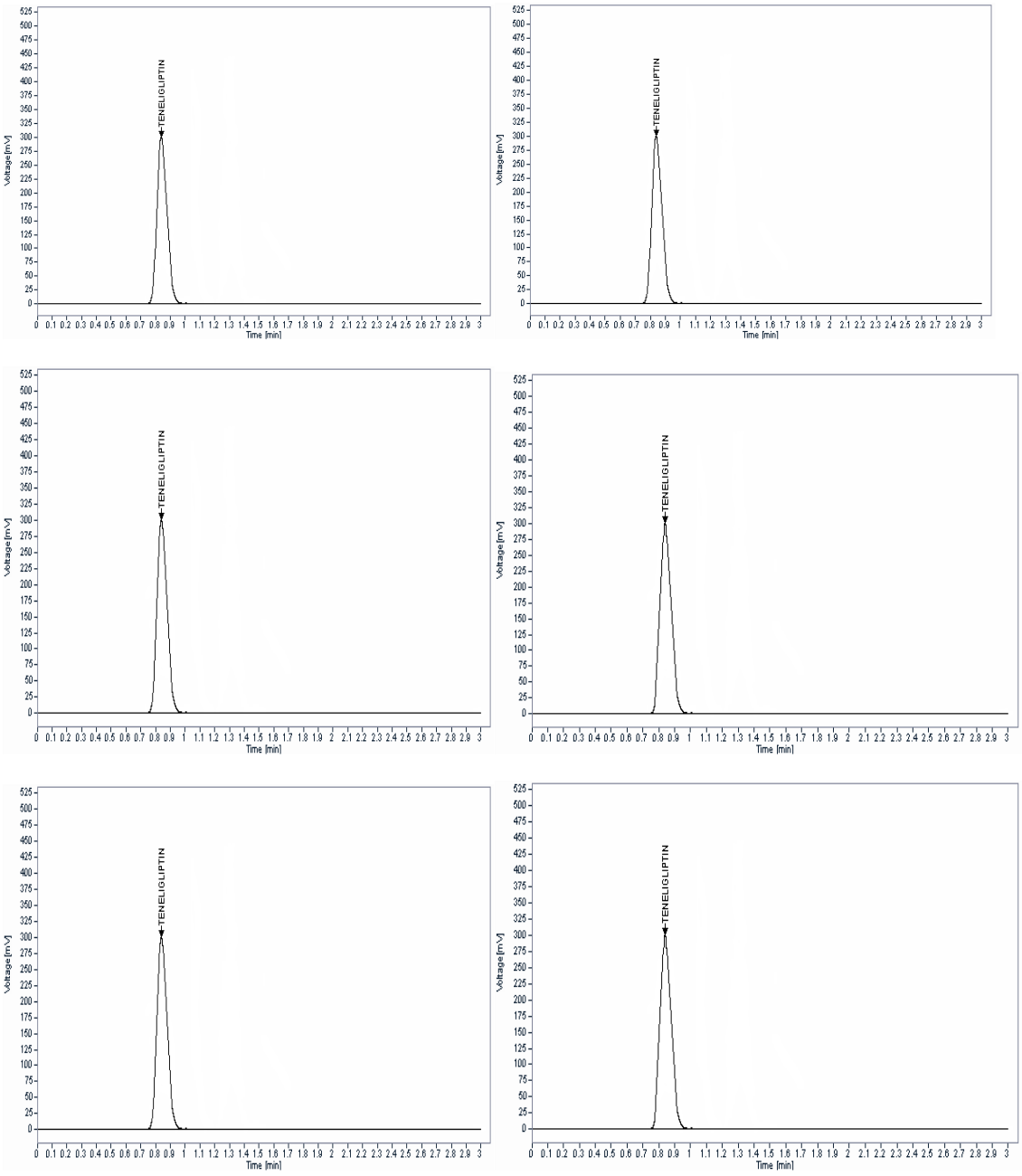
Graph No- 23

HIGHFLOWRATE (0.6 ml) STANDARD CHROMATOGRAMS



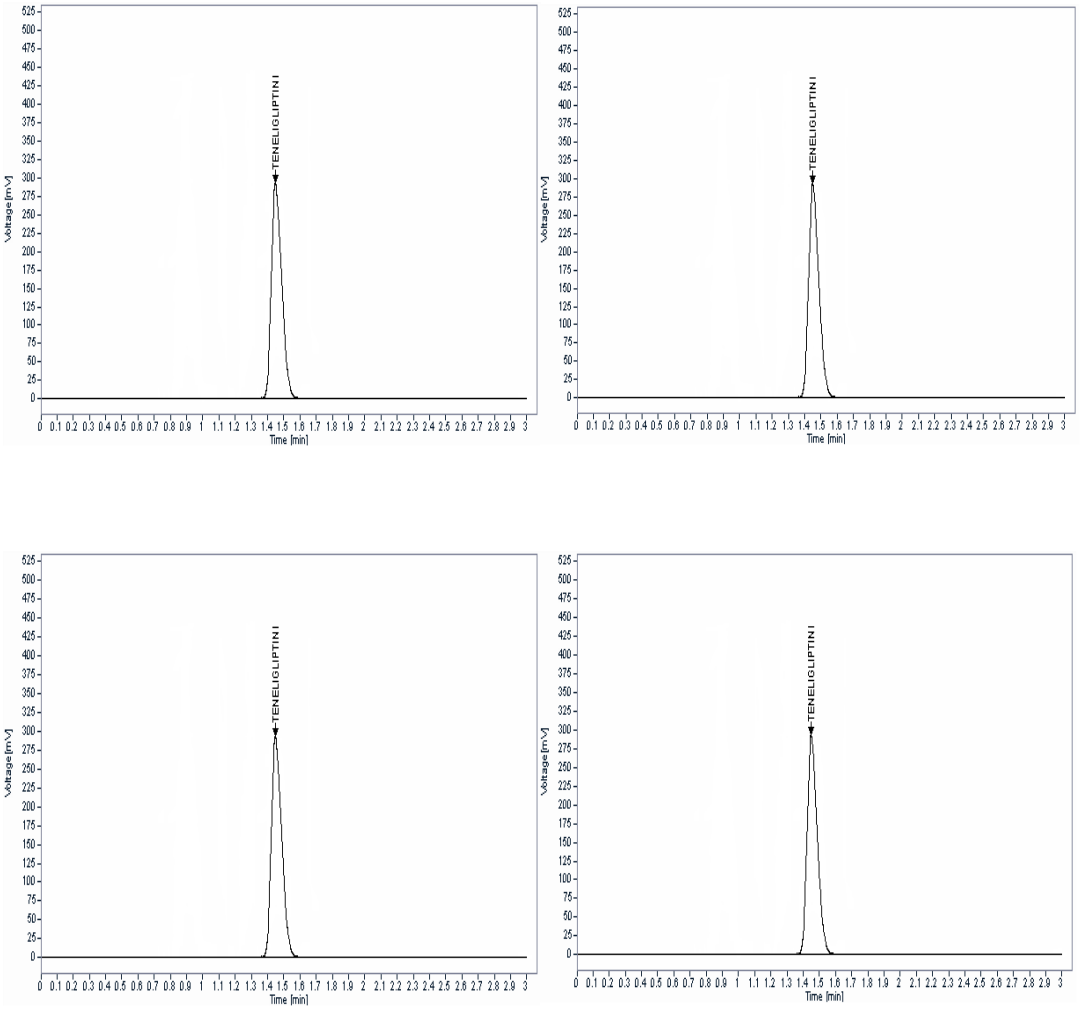
Graph No- 24

HIGH FLOW RATE (0.6 ml) SAMPLE CHROMATOGRAMS



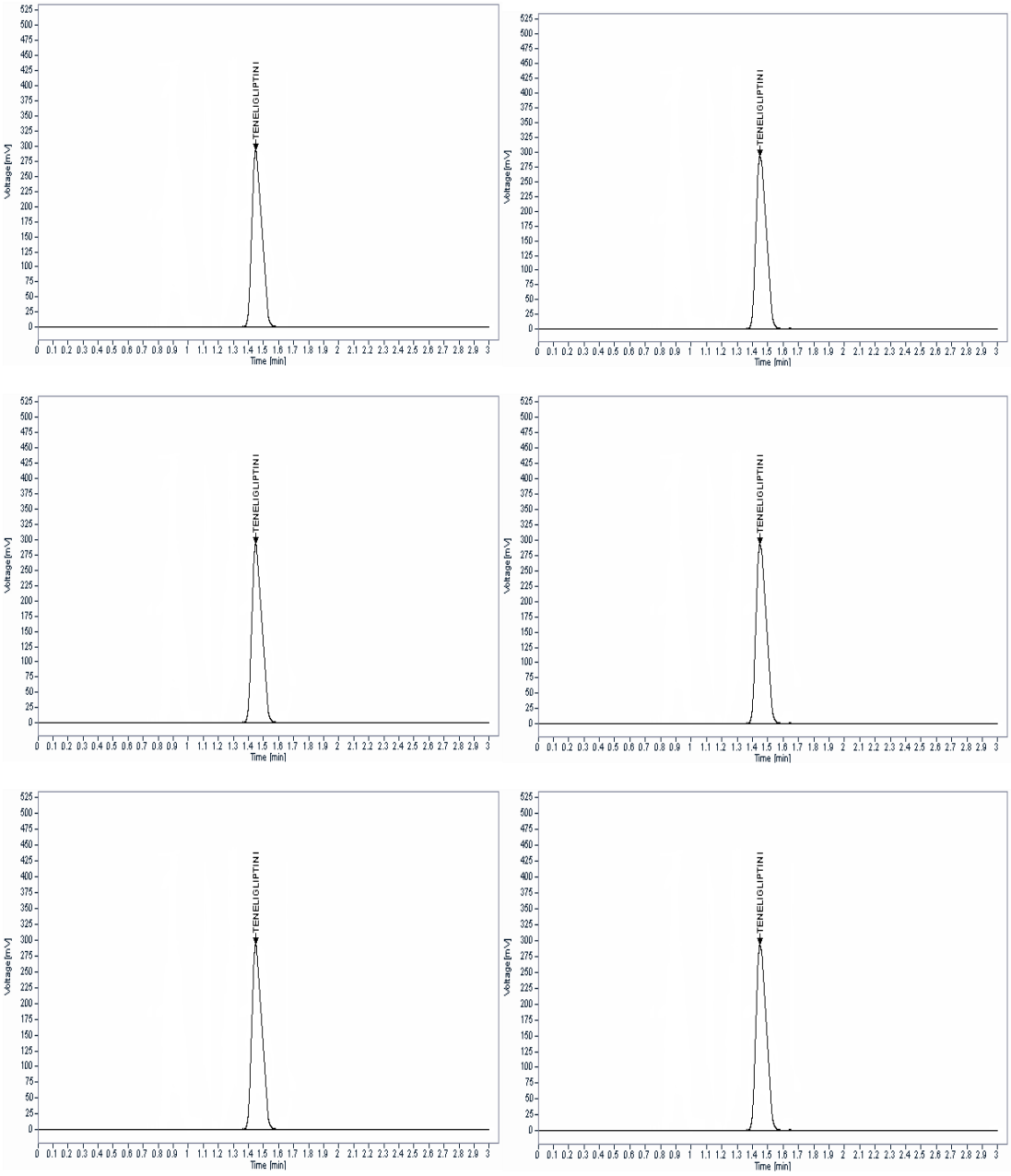
Graph No- 25

LESS ORGANIC RATIO STANDARD CHROMATOGRAMS



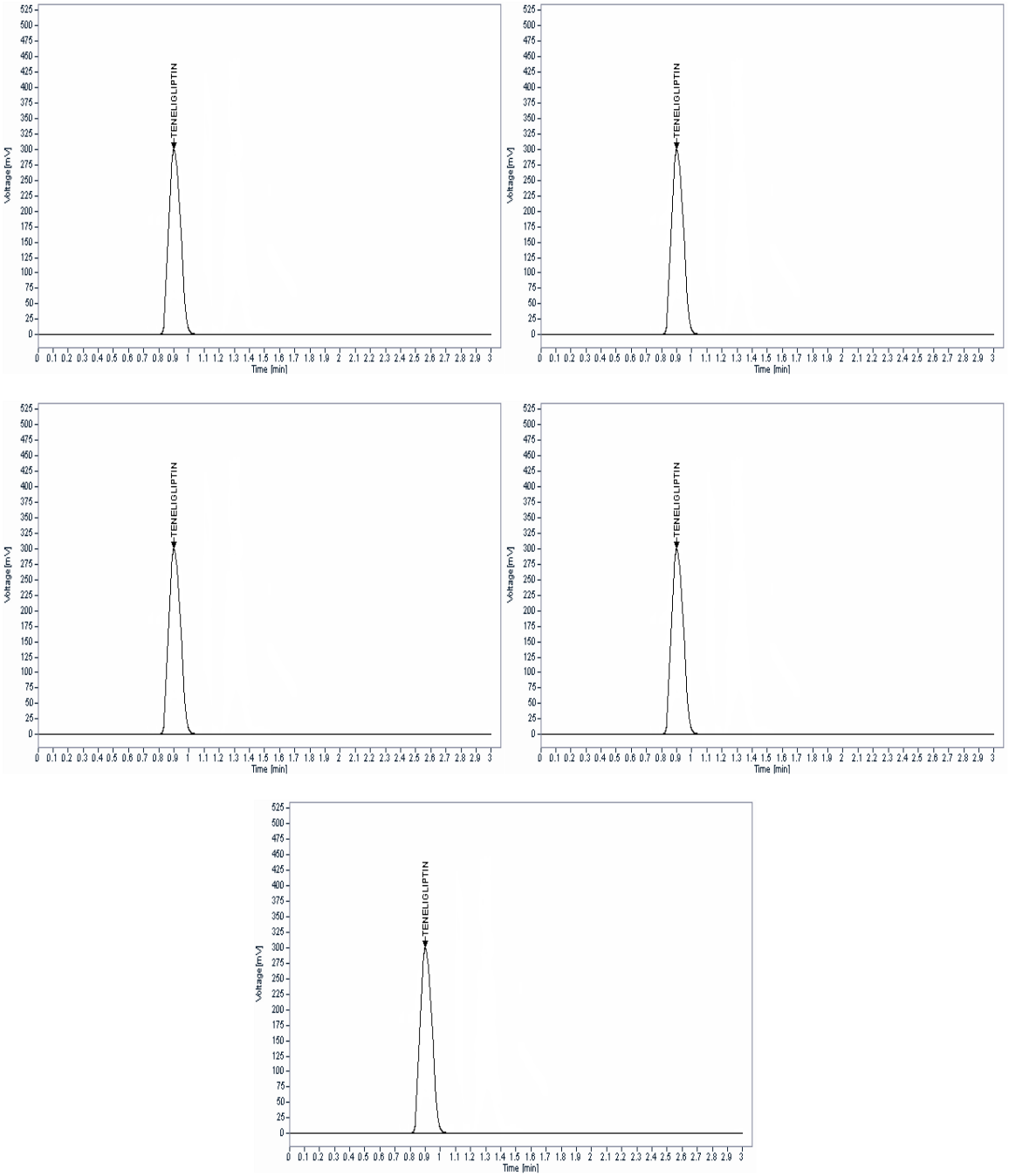
Graph No- 26

LESS ORGANIC RATIO SAMPLE CHROMATOGRAMS



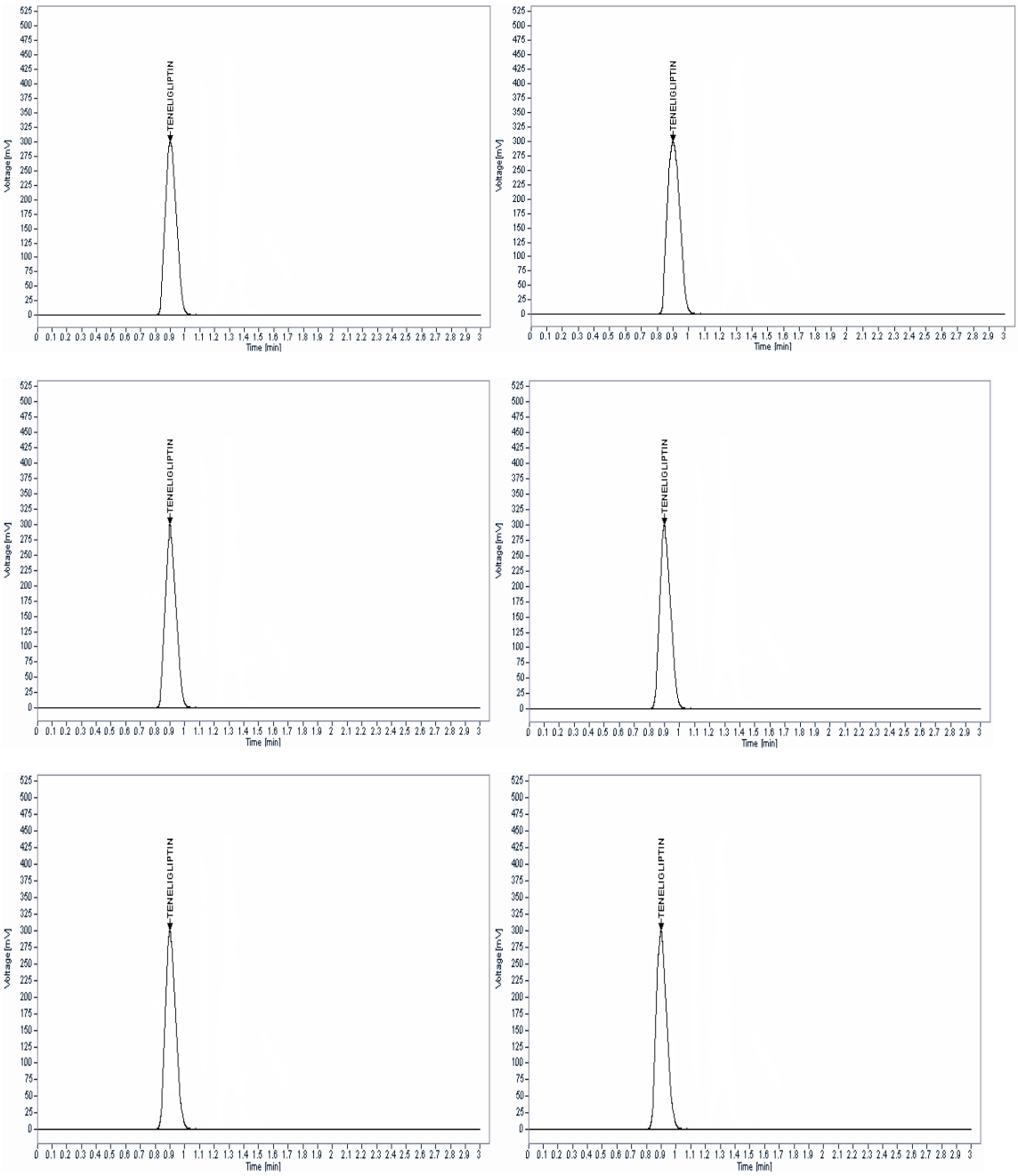
Graph No- 27

MORE ORGANIC RATIO STANDARD CHROMATOGRAMS



Graph No- 28

MORE ORGANIC RATIO SAMPLE CHROMATOGRAMS



Graph No- 29

RESULTS AND DISCUSSION

The new analytical method for the UHPLC method was established for Teneligliptin then optimized and then applied on pharmaceutical dosage forms.

Various mobile phase systems were prepared and used to provide an appropriate chromatographic separation, but the proposed mobile phase comprising of Buffer and Acetonitrile in the ratio 50: 50 gave a better resolution and sensitivity.

The detection was carried out by using UV detector at 245nm using Phenomenex Luna C18; 50 mm x 3.0 mm,3µm .Among the several flow rates tested, the flow rate of 0.5 ml was found to be the best for Teneligliptin with respect to retention times and theoretical plates.

The retention time is 1.02 for Teneligliptin. The asymmetry factor or the tailing factor was found to be 1.0 for Teneligliptin, which indicates symmetrical nature of the peak

System suitability parameters such as retention time, tailing factor, capacity factor and number of theoretical plates were calculated. The number of theoretical plates was found to be around 2100 for Teneligliptin, which indicates efficient performance of the column.These parameters represent the specificity of the method.

Linearity range was evaluated by the visual inspection of plot of peak area as a function of analyte concentration and the corresponding calibration graphs were shown in figure and results are shown in table.

From the linearity studies, the specified concentration range was determined. It was observed that Teneligliptin was linear in the range of 50% to 150% for the target concentrations.

The validation of the proposed method was verified by system precision and method precision. The %RSD for system precision and method precision of Teneligliptinwas tabulated.

The validation of proposed method was verified by recovery studies. The percentage recovery range was found to be within the limits for Teneligliptin. This is a good

index of accuracy, specificity and repeatability of the method. The obtained results were tabulated.

Placebo interference studies were made by injecting placebo alone, then the standard and the placebo along with the standard. They did not show any interference of placebo at the RT of the analyte peak

Robustness studies were made by varying the flow rate and also by performing filter validation studies on to types of filters. The analytical data and results for filter validation were tabulated, hence the developed method was found to be robust.

Study of ruggedness was made by conducting the study on different system and by two analysts. The results were found to be in limits and were tabulated and hence the developed method is found to be rugged.

Summary & Conclusion

A simple, reproducible and efficient reverse phase Ultra high performance liquid chromatography (RP-UHPLC) method has been developed for estimation of Teneligliptin in its tablet dosage form. Separation was done by using mobile phase consists of Mixture of 1-OctaneSulphonic acid sodium salt (pH adjusted to 3.5 ±0.05 with Orthophosphoric acid): Acetonitrile (50:50,v/v). Chromatography separations were carried out on Phenomenex Luna C18 column (50 X 3.0mn; 3µm) at a flow rate of 0.5ml/min and UV detection at 245nm and the retention time for Teneligliptin is 1.02 minutes. The linear dynamic response was found to be in the concentration of 50µg-150µg/ml. The slope, intercept and Correlation coefficient was found to be 0.9996 respectively. The percentage recovery of Teneligliptin was found to be 99.50 - 100.06%. Proposed methods were found to be simple, accurate, precise and rapid and could be used for routine analysis.

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